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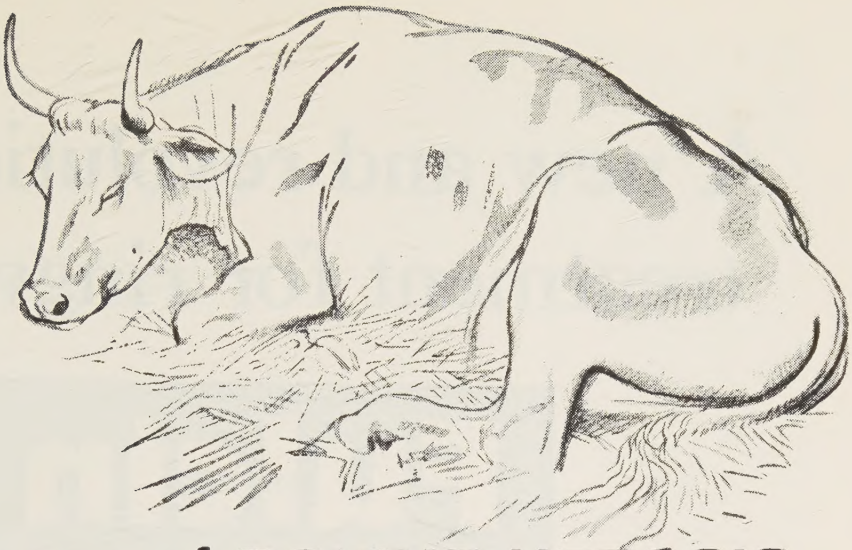

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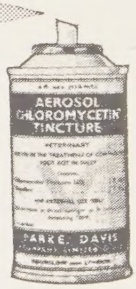

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VETERINARY REVIEWS AND ANNOTATIONS

Volume 6 October, 1960 *Part 2*

Some Biochemical Aspects of Ketosis with particular reference to cattle

BY

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INTRODUCTION

The volume of publications on ketosis and related topics, is large and expanding rapidly. In this survey no attempt has been made to cite all the relevant material, much has been deliberately omitted, doubtless even more has been overlooked and no attempt has been made to establish priority of discovery.

It has been necessary to refer frequently to experimental results in laboratory animals, since fundamental studies with farm animals have been comparatively few. It seems fairly probable that conclusions drawn from experiments with other species can be applied to the ruminant condition, but because of possible species differences this must be done with caution. Fortunately, it is likely that such differences will be quantitative rather than qualitative.

Ketosis is a condition in which the level of the ketone (or acetone) bodies in the body fluids is elevated. The ketone bodies are generally considered to consist of beta-hydroxy-butyrate ($CH_3.CHOH.CH_2.COOH$),

The following abbreviations are used:- CoA (or CoA.SH) = Coenzyme A; ATP = Adenosine triphosphate; AMP = Adenosine monophosphate; PP_I = Inorganic pyrophosphate; FAD = Flavin adenine dinucleotide; $FADH_2$ = Reduced flavin adenine dinucleotide; DPN = Diphosphopyridine nucleotide; DPNH = Reduced diphosphopyridine nucleotide; TPNH = Reduced triphosphopyridine nucleotide; NEFA = Non-esterified fatty acids.

acetoacetate ($CH_3.CO.CH_2.COOH$), and acetone ($CH_3.CO.CH_3$). To these, according to Robertson et al. (1950), should be added iso-propanol ($CH_3.CHOH.CH_3$), which they found in samples from cases of ruminant ketosis.

It will be seen that only acetone and acetoacetate contain the ketonic group (-CO-). The others are therefore not ketones in a chemical sense. However, beta-hydroxybutyrate is clearly closely related to acetoacetate, and iso-propanol to acetone and interconversions can occur in vivo (e.g. Thin & Robertson, 1953; Thin et al., 1959). There is abundant evidence that the metabolism of all four compounds follows certain common pathways, and that a change in the concentration of one leads, generally, to changes in the concentrations of the others.

To describe these compounds, we prefer to use the term "ketone bodies" rather than "acetone bodies". "Ketone" gives a better impression of a group of related compounds than does "acetone", since acetone is merely the trivial name for dimethyl-ketone which is only one member of the ketone homologous series.

In a similar way, "ketonaemia" (and "ketonuria") is preferred to "acetonaemia" (and "acetonuria") when the level of total ketone bodies in blood (or urine) is intended. We use "acetonaemia" (and "acetonuria") only to describe the level of preformed acetone. In practice, preformed acetone is frequently determined together with acetoacetate. The sum of these two ketones has been described by some German workers as "Gesamtaceton". For this we use a portmanteau word "diacetonaemia", which while etymologically unsound is of considerable utility.

To avoid confusion with this we use, for the concentration of aceto-acetate (diacetate), the term "acetoacetaemia" rather than the more concise "diacetaemia".

Some authors use "ketonaemia" in the sense of an elevated blood level of the ketone bodies but since blood from perfectly normal animals contains ketone bodies, this may lead to confusion. Elevated blood levels are better referred to as "hyper-ketonaemia" (similar considerations should, of course, apply to the use of "acetonaemia" instead of "hyper-acetonaemia"). As an analogy, few biologists use "glycaemia" to describe elevated blood sugar levels: "hyperglycaemia" is preferred.

The use of an expression such as "the ketonaemic animal", instead of "the hyperketonaemic animal", seems unlikely to lead to confusion; however, "ketotic" offers an alternative which seems quite unobjectionable.

This question of terminology is of some little importance. A semantic "iron-curtain" between clinicians and biochemists is undesirable. The consequence of such a barrier has already been seen in the case of the terms "acid" and "base" used in opposite ways by clinicians and chemists. In this connexion it should be emphasized that "ketosis" and "acidosis" are not synonyms. Either condition may occur in the absence of the other. Failure to recognize this has led to statements such as "Sampson quotes that alkalosis does have a ketogenic effect. Johnson contradicts himself by stating that propionic acid acts as an antiketogenic agent, and that the same acid when given by stomach-tube causes acidosis. We must note the contradictory statement that alkalies as well as acids, act as ketogenic agents, and that propionic acid is both ketogenic

and antiketogenic" (our italics). This quotation comes from a paragraph with the heading "Controversial Conclusions".

Unfortunately the use of "acetonaemia" for "ketosis" is too firmly embedded in the literature to be eradicated. However, it is felt that a clear indication should be given if the word is used in this way, for example the first reference could read "acetonaemia (ketosis)".

Even if this usage must be accepted there is no need, or justification, for writers to perpetrate further offences against communication. One has gone so far as to distinguish between "ketosis", an elevated ketone body level in blood and urine, and "acetonaemia", which was defined as a ketosis accompanied by clinical signs. It is felt that such distinctions cannot be deprecated too strongly. The distinction would be justified only if the clinical signs correlated with the level of pre-formed acetone, and not with that of total ketones. Evidence for this is lacking. Others have written "ketosis (was) established clinically without blood ketones being above normal". This makes sense to a reader accustomed to veterinary usages, but it is a contradiction in terms to the average biochemist. Certain clinical conditions in the ruminant, particularly in the cow in early lactation, and the ewe in late pregnancy, are generally accompanied by hyperketonaemia, and are commonly described as "ketosis". There have been claims that these conditions can exist in the absence of elevated ketone levels (the evidence for this need not be regarded as definitive). If, however, these claims are valid, then the answer is surely to rename the conditions, rather than redefine "ketosis" as clinical conditions seen in two species of ruminants ?

In the sheep this is readily done; the terms ovine "pregnancy toxaemia", "pregnancy disease" or "twin lamb disease" are quite firmly established as alternatives to ovine "ketosis", and seem generally preferable. In the cow the position is less good. Names such as bovine "post-parturient dyspepsia" are not entirely satisfactory. It has been suggested that the bovine condition should be renamed "hypoglycaemia". However, there have been reports of clinical cases with normal blood sugar levels (Boddie. 1935; Sampson & Bowley, 1941) and, contrariwise, of marked hypoglycaemia without clinical signs (Shaw, 1946b; Shaw & Leffel, 1949).

For the purposes of this review we are not particularly concerned with whether or not ketosis is an invariable concomitant of certain clinical conditions. It is enough that severe ketosis is of widespread occurrence in ruminants. Further, even if ketosis is not harmful per se, it undoubtedly reflects an underlying metabolic disturbance which is almost certainly unbeneficial.

It would seem possible that, under certain conditions, this underlying disturbance, while producing clinical signs, may not actually evoke frank ketosis. Further it seems possible, even probable, that ketosis in ruminants may have more than one cause. If this is so, then the tendency towards clinical signs unaccompanied by ketosis may be greater in some cases than in others. Conversely, circumstances may exist, where a relatively mild underlying disturbance, insufficient to evoke clinical signs, may be enough to produce significant ketosis. Individual variations may also be important in these respects.

In any event it is to be hoped that a study of ketosis will produce

evidence as to the nature of the primary metabolic change(s), so providing a more rational basis for prevention and treatment.

GENERAL CONSIDERATIONS

The ketone bodies are normally present in small amounts in the blood, and frequently also in the urine. It is known that normal extra-hepatic tissues are capable of utilizing ketones. Therefore it is likely that there is normally both some production and utilization of these metabolites, but perhaps at rather a slow rate. It should, however, be noted that Crandall et al. (1940) claim that ketones are not produced by the normal dog.

Ketosis would result if production was increased, or if utilization was decreased. Changes in excretion may also play a part. Evidence for decreased utilization of ketones is scanty. Moreover decreased excretion is unlikely in most ketotic conditions. Ketosis may therefore be regarded as mainly the result of increased ketogenesis. It should be admitted that there may be exceptions to this, the ketosis of alloxan diabetes may be due, in part, to decreased ketone utilization (Beatty et al., 1959). These workers found normal ketone utilization in fasting ketosis, so that, in this case at least, increased production is indicated. Moreover much other evidence exists for increased ketogenesis in ketotic states.

Some factors affecting ketone body levels in animals

Elevated ketone body levels in blood (and urine) are found during starvation (Cori & Cori, 1927; Crandall, 1941; Stark & Somogyi, 1943), and on diets low in carbohydrate, and high in fat and/or protein (Wigglesworth, 1924; Anderson & Anderson, 1927; Burn & Ling, 1928; McClellan & Toscani, 1928; Chamberlin et al., 1937).

Ketosis also occurs during severe muscular exercise (Courtice & Douglas, 1936; Gemmill, 1934; Passmore & Johnson, 1938), on exposure to a cold environment and during reduced fluid intake (Sargent et al., 1958; Johnson et al., 1958), after surgical trauma (Elman & Weichselbaum, 1951; Neptune, 1956), and X-irradiation (Kikuchi, 1931 but compare Ontko, 1960), during artificial fever (Somogyi & Kirstein, 1940) and exposure to reduced atmospheric pressure (Evans, 1936), and even during "stress" (Hinkle et al., 1951).

Various forms of alkalosis lead to ketosis (Davies et al., 1920; Butts et al., 1934–35; Engel, 1958).

Increased ketones are also found after the administration of ammonium chloride (Nichols & Nichols, 1958); phloridzin (Goldfarb et al., 1934), alloxan (Lukens, 1948), malonate (Recknagel & Potter, 1951), fluoroacetate (Cole et al., 1956) fluorocitrate (Gal et al., 1954), alpha, alpha'-dipyridyl (Hara, 1955; Takeda & Hara, 1955), and nicotinic acid (Janes & Myers, 1946; Janes & Brady, 1949).

Many of these conditions are associated with reduced, or inadequate, carbohydrate metabolism. The ketosis of diabetes is well known, and is also generally attributed to decreased carbohydrate metabolism.

Numerous hormonal effects on ketosis have been described. Anterior pituitary extracts have been found to be ketogenic (Burn & Ling, 1928, 1930; Anselmino & Hoffman, 1931; Best & Campbell, 1936; Greenbaum & McLean, 1953; Greenbaum, 1953). The liver is essential for the response (Mirsky, 1936). A direct pituitary effect not mediated by a "target" endocrine gland, seems likely (Shipley & Fry, 1942; Bennett et al., 1948; Engel & Engel, 1954, 1958; Gillman et al., 1958).

However, endocrine interactions do occur. Thus Tepperman & Tepperman (1951) found that adrenocorticosteroid was necessary for the ketogenic response to pituitary growth hormone. Others have found that corticosteroids inhibit fasting ketosis (Kinsell et al., 1951; Engel & Engel, 1954) and cold-stress and fluoroacetate ketosis but not ketosis due to insulin hypoglycaemia or to oxycel adrenocorticotrophin (Engel & Engel, 1958).

Best & Campbell (1936) found thyroid hormone to be essential for the ketogenic response to anterior pituitary extract. This contrasts with the work of Funk (1933), but the discrepancy is perhaps due to differences in time after thyroidectomy. Bondy & Wilhelmi (1950) and Gillman et al. (1958) also implicated the thyroid in the control of ketone metabolism but Butts et al. (1934) failed to demonstrate a ketogenic response to thyrotropic hormone.

The pancreas is probably involved also, either through insulin (Campbell, 1922; Tepperman et al., 1952; but contrast Shipley & Humel, 1945), or some other factor (Thorogood & Zimmermann, 1945; Stewart & Roitman, 1953). This latter factor is either the pancreatic hyperglycaemic–glycogenolytic substance (Kalant, 1954), or some still undetermined material (Foà & Weinstein, 1951; Foà, 1954).

Sex differences in ketone body metabolism have been reported in a number of species (Heinbecker, 1928; Deuel & Gulick, 1932; Hawley et al., 1933; Butts & Deuel, 1933; MacKay et al., 1941; Friedemann, 1942; Pearce, 1953). The differences seem to be due to the sex hormones (Chamberlin et al., 1937). These observations are possibly of importance for ruminant ketosis since in cattle and sheep, ketosis occurs predominantly when large changes in sex hormone balance are taking place. It is of interest that oestrogens seem to have an effect on the pituitary (Hohlweg, 1934; Wolfe & Chadwick, 1936; Bradbury, 1947). One consequence seems to be increased blood adrenocorticotrophin activity (Gemzell, 1953) and adrenal hypertrophy (Korenchevsky & Dennison, 1934; Selye & Collip, 1936). Prolactin, under certain conditions, is diabetogenic (Houssay & Anderson, 1949; Sinkoff & de Bodo, 1953), and according to Grant & Taylor (1952) citrate synthesis is inhibited by progesterone and certain other steroids. These observations suggest possible mechanisms by which sex hormones could affect ketosis, but the exact method remains undetermined.

Of subsidiary interest are observations that age (Wick & MacKay, 1940), time of year (Cori & Cori, 1927; Burn & Ling, 1928), and time of day (Hubbard & Wright, 1924; McClellan & Toscani, 1928) can all affect ketone metabolism.

Finally considerable species differences exist in susceptibility to ketosis. Man develops fasting ketosis fairly readily but the dog, cat, pig,

steer, goat, guinea-pig, rat and rabbit seem more resistant (Geelmuyden, 1901; Allen, 1923; Friedemann, 1926–27; Carpenter, 1927). The mature ruminant is generally resistant to starvation ketosis during non-productive phases, but is very susceptible during early lactation, and late pregnancy (Sjollema & van der Zande, 1923; Hutchinson & Morris, 1936; Thin & Robertson, 1953).

Strain differences are also known for the rat (Tidwell & Treadwell, 1946), while Heinbecker (1928) found Eskimoes to be more resistant than Europeans to the development of ketosis.

METABOLIC BALANCE

Ways in which ketosis may develop can be better appreciated if the metabolic reactions concerned in ketone production and utilization are first discussed.

The general pattern of intermediary metabolism is shown in Figure 1. Many intermediary reactions have been omitted in this diagram, and it should be pointed out that a particular tissue may not be capable of all the reactions shown. Further, certain of the reactions which are shown as reversible, for example, pyruvate $\rightleftharpoons$ triosephosphate, may involve different enzyme systems in the different directions (Krebs, 1954; Weinman et al., 1957; but contrast Krimsky, 1959).

Some metabolic peculiarities of ruminants

Although ruminants consume diets rich in carbohydrate, there is abundant evidence that ruminal digestion makes available for absorption a mixture of volatile fatty acids (Tappeiner, 1884; Mangold, 1934; Barcroft et al., 1944; Phillipson, 1955).

On a maintenance ration there is good reason to believe that virtually no digestible carbohydrate escapes such breakdown (Heald, 1952; McClymont, 1951). However, on a production ration it is possible that some carbohydrate escapes ruminal fermentation, and is available for intestinal digestion. The apparent inefficiency of such digestion in ruminants (Reid, 1952, 1958; Clark & Weiss, 1955) is somewhat against this idea, but does not exclude it.

A more comprehensive discussion of the carbohydrate supply of the ruminant, and of carbohydrate metabolism in general, is to be found in a recent review by Lindsay (1959). He gives estimates of volatile fatty acid production in the rumen.

Acetate seems to be the major volatile fatty acid produced in the rumen. In consequence of rapid metabolism of propionate and butyrate, by the splanchnic area, very little of these acids passes into the peripheral circulation. Acetate seems to be the principal free volatile fatty acid in peripheral blood (McClymont, 1949; Reid, 1950).

Recently Shibata & Umetsu (1957) have reported large amounts of combined volatile fatty acids in peripheral blood; up to 40 to 60 mg. per 100 ml. blood were observed, that is five to ten times the level of the free volatile fatty acids. It is not yet known whether acetate is also the principal combined volatile fatty acid. Indeed the entire significance of this form of fatty acid remains to be determined.

Major metabolic pathways in animal tissues

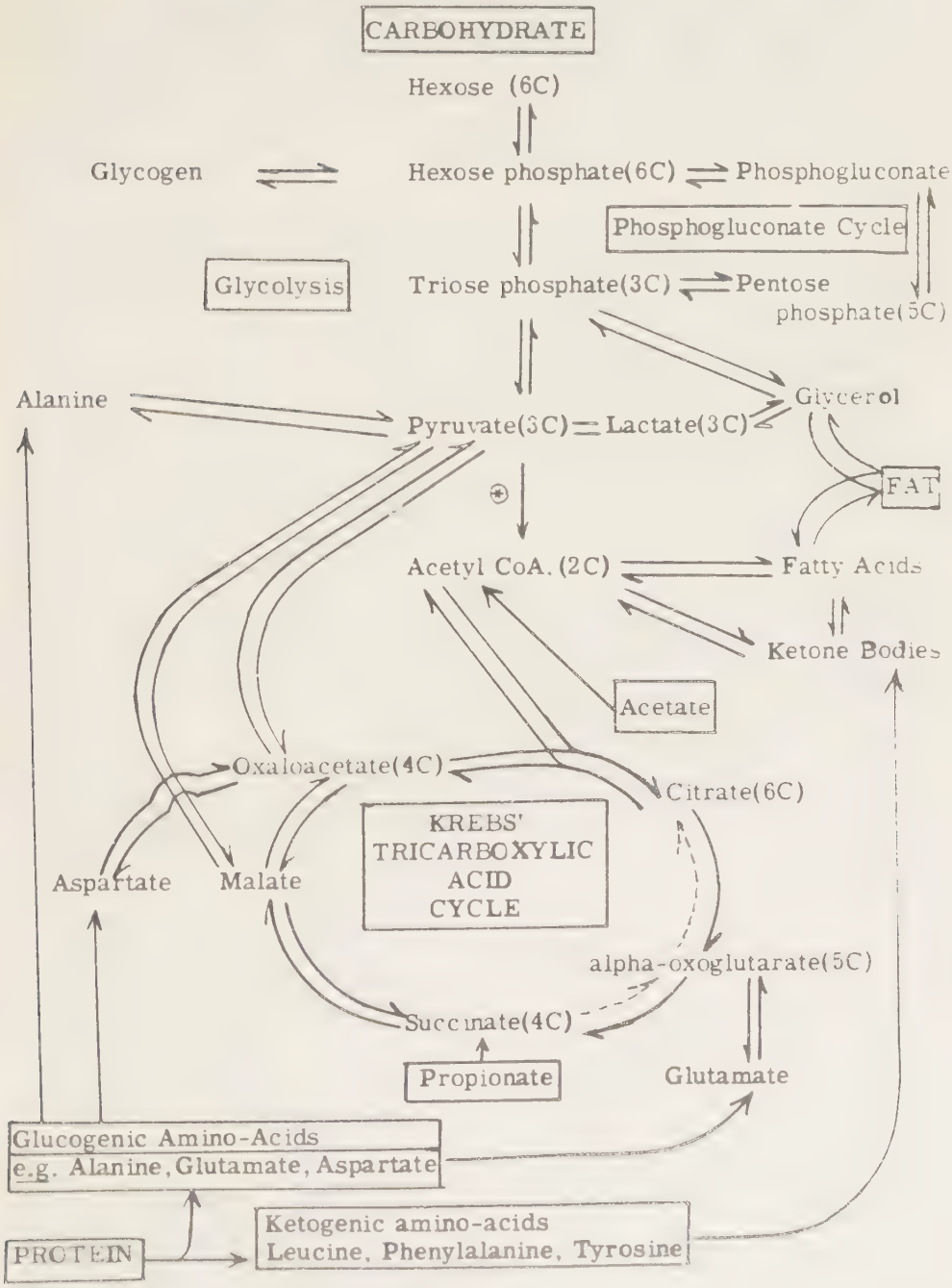

Figure 1.

⊛ N.B. The single arrow here is very important.

There is every reason to believe that the tricarboxylic acid cycle is the main route for oxidation of acetate. Acetate oxidation has been demonstrated in a wide variety of tissues, including heart muscle (Lorber et al., 1946; Pearson et al., 1949), skeletal muscle (Lifson et al., 1951), lung, liver and kidney (Pardee et al., 1950), and mammary gland (Folley & French, 1950; Popják et al., 1951; McClymont, 1951). It has been reported that acetate is not oxidized by brain (Long & Peters, 1939; Elliott et al., 1942).

Butyrate can also be oxidized via the tricarboxylic acid cycle, although with perhaps a greater chance of intermediate ketone body formation.

However, in the case of propionate, the other principal volatile fatty acid, metabolism follows a rather different course. It is fairly certain that this acid is metabolized via succinate to malate and oxaloacetate, and to phosphopyruvate (Flavin et al., 1955; Beck & Ochoa, 1958; Katz & Chaikoff, 1955a).

Some evidence also exists for metabolism of propionate via acrylate to lactate (Huennekens & Mahler, 1950).

In any event phosphopyruvate (or lactate) should be potentially convertible to pyruvate and to glucose. Pyruvate may be decarboxylated to yield acetyl coenzyme A, and this can subsequently be oxidized via the tricarboxylic acid cycle. However, while the evidence is by no means definitive, there seems a chance, for the ruminant, that the reaction pyruvate → acetyl coenzyme A may be rate limiting (cf. Folley, 1956). Possible evidence for this would be that:-

(1) The glucose tolerance of ruminants (at least on high fibre diets) is of "diabetic" type (McCandless et al., 1948; Holmes, 1951; Reid, 1958).

(2) Ruminants, after glucose administration, show a slower rise in blood pyruvate than do other species, but having once risen, the elevated level persists longer (Jarrett & Potter, 1954).

(3) Much evidence exists that a certain minimum amount of fibre is necessary, in the diet of lactating cows, for optimal butter-fat production. Dietary fibre may be replaced by acetate but not by propionate (Stoddard et al., 1949; McClymont, 1950; Tyznik & Allen, 1951; Van Soest et al., 1954; Van Soest & Allen, 1959).

(4) In ruminant mammary gland acetate is utilized much more effectively for fatty acid synthesis than is carbohydrate (Balmain et al., 1954).

(5) Black et al. (1957) have concluded that, in intact cows, between 40 and 80% of glucose metabolism occurred via the "phosphogluconate oxidative pathway", that is, did not involve intermediate formation of acetyl-coenzyme A. It should be conceded that their results are possibly susceptible to other explanations. However, their claim is by no means unreasonable. Work with the rat has suggested that the phosphogluconate pathway may be particularly important in liver and mammary gland (Murphy & Muntz, 1957; Agranoff et al., 1954; Abraham et al., 1954; Glock & McLean, 1954; Glock et al., 1956). It is of interest that these two organs are likely to be of paramount importance in the development of bovine ketosis. Somewhat unfortunately Glock et al. found only low phosphogluconate pathway enzyme activities in ovine mammary gland. Assays on bovine udder would be of great interest.

Even if phosphogluconate activity should prove to be low in the bovine gland, this need not lead to the idea that the pathway is unimportant. It is to be expected that the relative extent of glucose oxidation should be smaller in ruminant tissues than in rat tissues, in consequence of relatively smaller carbohydrate supply. Relatively lower enzyme activities might then be capable of dealing with the major part of carbohydrate oxidation in ruminant mammary gland.

Despite difficulties of interpretation, and despite also some conflicting evidence, there may be a case for giving consideration to two possibilities:

(1) That the reaction pyruvate→acetyl coenzyme A may be rate limiting in ruminant mammary gland (and perhaps also in other organs);

and

(2) That carbohydrate oxidation in the ruminant, occurs mainly by way of the phosphogluconate pathway. An important objection is the failure to demonstrate marked phosphogluconate activity in muscle. A very large part of the total body catabolism is undoubtedly attributable to the muscles. If this fraction of the total catabolism accounts for much of the total carbohydrate oxidation then statement (2) should be revised to read:

(3) That carbohydrate catabolism in the ruminant mammary gland and liver occurs mainly by way of the phosphogluconate pathway.

It is possible, however, that muscle does not normally utilize much carbohydrate (e.g. Andres et al., 1956).

Reports of high activities for the enzymes of the phosphogluconate pathway in bovine adrenal cortex (Kelly et al., 1955) are of interest in view of the probable participation of hormonal mechanisms in ketosis. This pathway has also been reported in pancreatic beta-cells (Field et al., 1960).

It would seem possible that the postulated defect in the conversion of pyruvate to acetyl coenzyme A may be related to the dietary supply of the ruminant. Thus in non-ruminants it has been found that glycosuria (Bernard, 1859; Hofmeister, 1890), and pseudo-diabetic glucose tolerance curves (Bang, 1913; Sevringhaus, 1925; du Vigneaud & Karr, 1925; Sweeney, 1927), occur when carbohydrate is administered after a period of fasting, or of high fat diet feeding. Moreover, in "starvation diabetes" the rise in blood pyruvate after glucose administration is slower, and more prolonged, than normal (Wollenberger & Linton, 1947). Much other evidence also exists that diet affects the direction and extent of carbohydrate metabolism (Mirski et al., 1938; Samuels et al., 1942; Lundbaek & Stevenson, 1948; Gilmore & Samuels, 1949; Wertheimer & Ben-Tor, 1950; Kerly & Ottaway, 1954). The general tendency of these experiments is to suggest that non-carbohydrate diets tend to favour glycogen synthesis, and to diminish glucose oxidation. A metabolic mixture rich in fatty acids may tend to produce a similar effect in ruminants, and a possible mechanism for such an effect would be decreased pyruvate decarboxylation. In this connexion it is of interest that, in rat liver, fasting was found to favour carboxylation of pyruvate to dicarboxylic acids (potentially convertible to glucose), while decarboxylation to acetyl coenzyme A was suppressed (Freedman & Graff, 1958).

Production of ketone bodies (ketogenesis)

(a) Site of origin. In the non-ruminant there is general agreement that the liver is the principal (perhaps only) source for the blood ketones (Embden & Engel, 1908; Chaikoff & Soskin, 1928; Goldfarb & Himwich, 1933; Jowett & Quastel, 1935; Mirsky, 1936). This does not imply that extra-hepatic tissues cannot form ketones, but merely that they do not usually release them into the blood. Extra-hepatic tissues are capable of utilizing ketones, and this might mask production. Ketogenesis in the kidney has, in fact, been demonstrated by Medes et al. (1945), and some evidence exists that it can occur in a wide range of tissues (Brady et al., 1951). It is therefore apparent that, in non-ruminants, the importance of the liver, as the source of the blood ketones, depends upon a greater rate of production than of utilization by this organ.

In ruminants an important role for the liver in the development of ketosis is suggested by the finding that valeric acid is ketogenic in ovine liver but not in kidney (Annison & Pennington, 1952). It has also been suggested that ketones may be produced in the rumen as a result of fermentations (Koffman, 1937; Boddie, 1949).

It is probable that volatile fatty acids produced in the rumen, are a significant source of ketone bodies. Catabolism by the liver would be expected to give rise to some ketones. Further butyrate (and to some extent acetate), may be metabolized by ruminal, reticular and omasal epithelium with the production of ketones (Pennington, 1952; Pennington & Pfander, 1957; Annison et al., 1957). However, McCarthy et al. (1956a) concluded that, in the ruminant, ketones are formed mainly by hepatic catabolism of long-chain fatty acids.

In lactating animals ketones may also be formed in the mammary gland (Terner, 1958).

(b) Precursors. Embden & Marx (1908), using the perfused liver, demonstrated ketogenesis from fatty acids, certain amino-acids, and pyruvate. Similar results have been obtained by later workers using a variety of techniques (Edson, 1935a, b; Cohen, 1937; Leloir & Muñoz, 1939).

There is general agreement that the main source of ketones is fatty acids. Ketogenic amino-acids also make a definite contribution. The position of pyruvate is less clear. Lehninger (1946a) found it to be ketogenic in malonate-poisoned liver mitochondria, but Weinhouse & Millington (1951) found that, although it was ketogenic in liver homogenates, it was not in liver slices. It would seem likely that, at least in normal animals, pyruvate makes little contribution to ketogenesis.

The ruminant's diet is normally low in fat but as a result of ruminal fermentations, the metabolic mixture made available to the animal contains very large amounts of the lower (volatile) fatty acids. Those fatty acids containing an even number of carbon atoms (e.g. acetate and butyrate) may be regarded as potentially ketogenic, while propionate (3C) is potentially glycogenic and antiketogenic.

(c) Ketogenesis from fatty acids. The basic observations on fatty acid oxidation, and ketone production, were made by Knoop (1904), who originated the theory of beta-oxidation. The history of developments in this field has been admirably outlined by Soskin & Levine (1946). A major advance was the "beta-oxidation-condensation hypothesis" of MacKay,

Wick & Barnum (1940) that long-chain fatty acids were broken down by beta-oxidation to two-carbon ("acetate") fragments, and that these were then capable of undergoing recondensation (in pairs) to give acetoacetate. The possibility of such a condensation had been demonstrated earlier by liver perfusion studies (Friedemann, 1913), and has been amply confirmed by isotopic methods (Swenseid et al., 1942; Weinhouse et al. 1944).

The hypothesis has subsequently undergone a slight revision. It is now believed that, while most of the fatty acid chain is broken down into two-carbon fragments, the terminal four-carbon ("omega") fragment is potentially capable of forming acetoacetate directly, without preliminary formation of two-carbon fragments.

A still more notable advance was the identification of the intermediary two-carbon fragment as acetyl coenzyme A (Lynen et al., 1951; Lynen & Reichert, 1951). Subsequently progress was made with the isolation of water-soluble enzymes involved in fat catabolism (Stadtman & Barker, 1949; Drysdale & Lardy, 1953; Wakil & Mahler. 1953; Green & Sanae, 1953; Coon et al., 1953; Goldman, 1953).

The modern view of the reactions involved is shown in Figure 2. It is to be noted that although all the reactions leading from fatty acids to acetyl coenzyme A have been shown to be reversible, yet, in certain cases the equilibria lie in the direction of catabolism, rather than of synthesis. Thus, although lipogenesis could theoretically result from direct reversal of beta-oxidation this is likely to be quantitatively unimportant. It is probable that separate enzyme systems are involved in fatty acid oxidation and in lipogenesis. Recently evidence for such a segregation of catabolic and anabolic pathways has become available (Gibson, Titchener & Wakil, 1958; Wakil et al., 1958; Brady, 1958).

It had earlier been demonstrated that, while diphosphopyridine nucleotide is involved in beta-oxidation, triphosphopyridine nucleotide is required for lipogenesis (Langdon, 1955, 1957). An important point of difference seems to be that triphosphopyridine nucleotide is required for the conversion of crotonyl coenzyme A to butyryl coenzyme A.

The possibility that the enzyme systems for anabolism and catabolism may have different loci within the cell, and the need for a different pyridine nucleotide coenzyme for synthesis compared with oxidation, may have important consequences for theories of ketosis, and will later be discussed further.

A highly simplified diagram (Fig. 3) shows the relationship between beta-oxidation and ketogenesis. It will be seen that obligatory formation of acetoacetate (defined as formation of acetoacetate without intermediate formation of acetyl coenzyme A) cannot exceed one mole of acetoacetate per mole of fatty acid catabolized. The actual value for obligatory formation could well be less than this, depending on the extent to which reactions (1) and (2) were successful in competing for acetoacetyl coenzyme A.

Any synthesis of acetoacetate in excess of one mole per mole of fatty acid catabolized must be due to condensation of acetyl coenzyme A to form acetoacetyl coenzyme A, with subsequent conversion of this to free acetoacetate (facultative formation). Ketogenic ratios greater than unity have in fact been frequently observed (Hurtley, 1916; Deuel et al., 1936;

Beta-oxidation of fatty acids, and some inter-relationships between ketone bodies

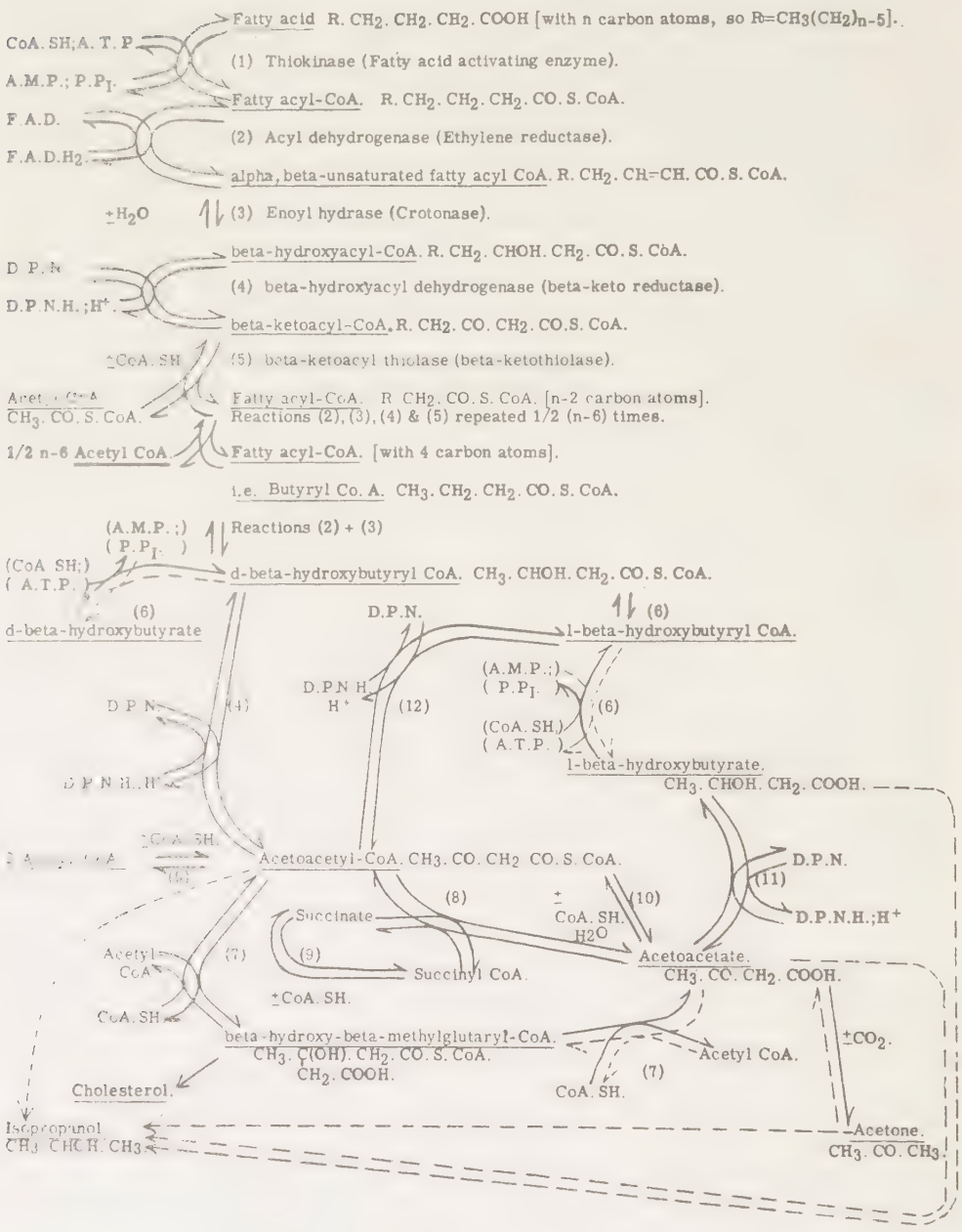

Figure 2.

Legend to Fig. 2 (facing)

Reactions which are not yet firmly established are indicated by broken arrows.

The enzyme nomenclature is that suggested by Beinert et al. (1956), but some older names are also given in parentheses.

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(1) At least three enzymes are known for this type of reaction, with differing acyl chain-length specificities (Lipmann et al., 1952; Mahler et al., 1953; Kornberg & Pricer, 1953a, b).
(2) At least three enzymes are known with differing specificities (Mahler, 1953; Hauge et al., 1956).
(3) Only one enzyme is known (Wakil & Mahler, 1954; Stern & Raw, 1954).
(4) The enzyme seems to have a broad specificity, C4–C18 (Lynen, 1954).
(5) At least two enzymes are known (Lynen & Ochoa, 1953).
(6) Lehninger & Greville (1953); Stern et al. (1955).
(7) Lynen et al. (1958).
(8) Stern et al. (1953).
(9) Kaufman et al. (1951).
(10) Stern et al. (1960).
(11) l-beta-hydroxybutyric dehydrogenase.
(12) Wakil (1955).

Obligatory and Facultative Ketogenesis

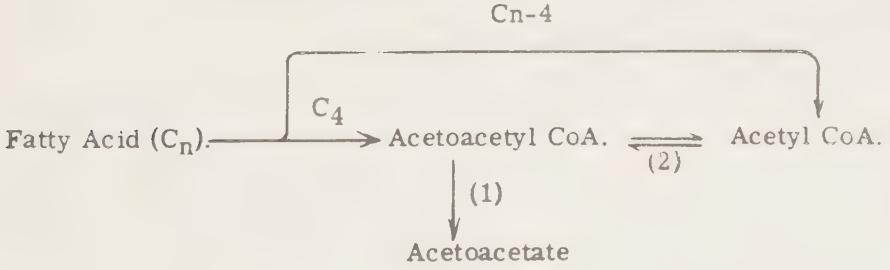

Figure 3.

Stadie et al., 1940). It is therefore very likely that condensation of acetyl coenzyme A plays a very important part in the development of ketosis.

The possibility of ketone formation from acetyl coenzyme A implies that any metabolite capable of forming this intermediary is potentially ketogenic. In view of the central position in metabolism occupied by acetyl coenzyme A_2 it would therefore seem that carbohydrates and

amino-acids, as well as fatty acids, should be able to form ketones. That this is so is clearly demonstrated by the study of Gurin et al. (1947). However, their work also indicates that the quantitative contribution of carbohydrate to ketogenesis must be quite small. It seems that it is only under conditions where fatty acid catabolism is appreciable that much ketogenesis occurs. The reason for this is by no means certain, but some attempt at explanation will be made later.

Utilization of ketone bodies

There is abundant evidence that many extra-hepatic tissues (mammary gland, muscle, kidney, heart, spleen, omentum, and possibly brain) are able to oxidize ketones rapidly and completely (Edson & Leloir, 1936; Barnes et al., 1940; Stadie et al., 1940; Shaw & Peterson, 1943; Shipley, 1944). It would appear that acetoacetate and beta-hydroxybutyrate are first converted to their coenzyme A complexes, and thence to acetyl coenzyme A (Fig. 2). Complete oxidation to CO_2 and water is then possible through the tricarboxylic acid cycle.

In contrast, it is clear that liver has a very limited capacity for ketone oxidation (Chaikoff & Soskin, 1928; Mirsky & Broh-Kahn, 1937; Quastel & Wheatley, 1933; Lehninger, 1946a; Weinhouse & Millington, 1951). Weinhouse and Millington found the rate of ketone oxidation in liver to be only 10% of that in kidney.

It would seem probable, therefore, that the predominant position of the liver, as a source of blood ketones (at least in non-ruminants), is to be related to its small capacity for ketone utilization and large capacity for ketone production.

There is no general agreement as to the extent of ketone metabolism in the normal animal. Crandall et al. (1940), using the London cannula technique, were unable to demonstrate hepatic ketogenesis in the fed, normal dog. In contrast Weinhouse and Millington found, in the fed, normal rat, a production, in vitro, of approximately 120 to 240 μM acetoacetate per 100 g. body weight per day. Even this estimate of production seems rather low.

Stadie (1945) calculated that ketone utilization could amount to as much as 3000 μM/100 g. body weight/day. There would seem a possibility either that in vitro hepatic ketogenesis does not accurately reflect in vivo production, or that the "pool" of acetoacetate added by Weinhouse and Millington to their liver slices inhibited ketogenesis (cf. Cohen & Stark, 1938). Alternatively it would seem necessary to suppose that normally ketone utilization is very slow.

The position is equally uncertain in abnormal metabolic states. Gameltoft (1949) concluded that, in the dog, up to 70% of the total peripheral metabolism, and all the peripheral fat metabolism, was accounted for by ketone oxidation after three days' fasting. Similar conclusions have been reached by others (Barnes et al., 1938; Shaw & Peterson, 1943).

On the other hand, Stadie (1940) calculated that in the diabetic human being, or the depancreatized cat, ketone oxidation could not account for more than one third of the total fat catabolism.

The possibility of gluconeogenesis from ketone bodies and fatty acids

Formation of carbohydrate from fatty acids, and their catabolites, has always been a controversial subject, and the question is still by no means settled.

Heller (1936) & Blixenkrone-Møller (1938), from studies of hepatic arterio-venous differences, concluded that fatty acids were capable of giving rise to carbohydrate. Haarman & Schroeder (1938) suggested that beta-hydroxy butyrate was able to form lactate. Lackey et al. (1947) found that intravenous administration of ketone bodies increased cardiac glycogen, but not liver or muscle glycogen. Ottaway & Sarkar (1958) found that acetoacetate produced an increase in glycogen in the perfused heart. The experiments of Meyer & Bow (1956) may be contrasted.

A possible pathway for conversion of ketone bodies to carbohydrate was suggested by the work of Sakami & Lafaye (1951) and Rudney (1952, 1954). They found that acetone is metabolized to a three-carbon glycolytic intermediary. 1,2-propanediol-1-phosphate appeared to be an intermediary.

There is little reason to doubt the reality of this conversion, but its quantitative importance is still uncertain. Acetone metabolism is known to be slow. However, as Rudney has pointed out, the process may be:

"Active acetoacetate" (acetoacetyl coenzyme A)
↓
"Active acetone"
↓
3C glycolytic intermediary (e.g. lactate or ? methyl glyoxal).

This consideration is reinforced by the fact that very large amounts of acetone are found in biological fluids during ketosis. Since acetone production from acetoacetate itself appears to be rather slow, it would seem possible that a large part of the acetone arises from acetoacetate in statu nascendi.

Another pathway for fatty acid gluconeogenesis would be the "glyoxylate cycle" (Kornberg & Krebs, 1957) which has been demonstrated in plants. However, certain key enzymes of this cycle do not seem to be present in animal tissues (Madsen, 1958).

The "Thunberg" condensation (2 "acetate" $\rightleftharpoons$ 1 succinate) would also afford a pathway. Seaman (1957) has reported the occurrence of this reaction in rat brain, heart, liver and muscle. However, he found that the condensation reaction rate was only 10% of the reverse reaction rate.

Kreuger (1950) has suggested that ketone bodies can form carbohydrate through intermediate formation of glycogenic amino-acids. In this connexion reference may also be made to experiments by Gibson, Laver & Neuberger (1958) and Elliott (1959).

Ellis (1952) has found that iso-propanol gives rise to carbohydrate in the perfused liver. It is of considerable interest that Thin & Robertson (1953) found this ketone body to be present in considerable amounts, in cases of bovine ketosis.

In view of these varied reports of gluconeogenesis from fatty acids and ketone bodies, it would seem quite likely that such conversions do occur. However, what is not yet established is whether the process makes a significant contribution to the carbohydrate supply of the animal.

Keto-antiketogenic balance.
Regulation of ketogenesis and ketone utilization

The mechanisms of ketone production and utilization having already been discussed, it remains to enquire how the two processes are normally balanced and under what conditions ketosis develops.

The so-called "Ketolytic hypothesis" maintains that ketosis develops when carbohydrate metabolism is suppressed, and that for ketone oxidation concomitant oxidation of carbohydrate is essential (Shaffer, 1923). This hypothesis springs from the observation that man develops ketosis if less than 10% of the calories produced from non-nitrogenous sources are derived from carbohydrate, or more than 90% from fat. This finding led to the concept that metabolism of a half molecule of glucose would be required to ensure the complete oxidation of one long chain fatty acid molecule (which was at that time assumed to form one molecule of ketone body). "Keto-antiketogenic formulae" of this type gained wide credence, but predictions from such a theoretical basis soon proved unreliable, and the formulae were found to have no application to species other than man.

In opposition to the concept of "Ketolysis" is the "Antiketogenic hypothesis" of MacCallum (1930), which maintains that ketosis occurs whenever fat catabolism is increased. Thus, for example, ketosis ensues if fat catabolism is increased to compensate for reduced carbohydrate catabolism. Further it is held that ketone oxidation is not coupled in an obligatory way to carbohydrate oxidation. Essentially, as pointed out by Edson (1936), this may be regarded as a hypothesis of substrate competition. Many proponents of the hypothesis have supposed that carbohydrate and fatty acids are in competition for the available oxygen.

(a) Ketolysis. The oxaloacetate deficiency hypothesis. The balance of opinion between these two major hypotheses was upset by the finding that oxaloacetate (a C_4 dicarboxylic acid, derived from carbohydrate) functioned catalytically in the oxidation of two-carbon fragments ("active acetate" or acetyl coenzyme A) derived from both carbohydrate and fatty acids. Further it was found that if oxaloacetate deficiency was induced experimentally, then ketosis ensued (Lehninger, 1946a; Recknagel & Potter, 1951; Cole et al., 1956; Gal et al., 1954). In recent years the hypothesis that ketosis is due to oxaloacetate deficiency has largely dominated the field. Indeed it is difficult to find a review in the recent veterinary literature which even considers an alternative explanation.

The hypothesis supposes that, if carbohydrate metabolism is suppressed, a deficiency of oxaloacetate occurs. Since this acid is required (in catalytic amounts) for the operation of the tricarboxylic acid cycle, a deficiency should lead to a slowing down of oxidation by this pathway. A major source of energy generation would thus be lost. It is

supposed that, in an attempt to compensate for this, fatty acid catabolism is increased. The process of beta-oxidation is associated with oxidative phosphorylation, so that energy may be generated in this way. However, if fatty acid catabolism is not carried further than the acetyl coenzyme A stage, much energy remains locked up in this compound. Consequently it is supposed that, when acetyl coenzyme A oxidation is blocked by oxaloacetate deficiency, a large and wasteful increase in beta-oxidation becomes necessary, in order to supply the energy required. Large amounts of acetyl coenzyme A are then generated, and it is supposed that, in order to free coenzyme A for further beta-oxidation, the acetyl moiety is diverted to ketone body (and sometimes cholesterol) synthesis.

This hypothesis has certain attractive features; however, it has always seemed incapable of explaining certain observations (Weinhouse, 1955; Pearce, 1953). For example, with the one possible exception of alloxan diabetes (Beatty *et al.*, 1959), there seems little satisfactory evidence that ketosis is associated with impaired extra-hepatic ketone utilization. Most studies have demonstrated normal or even elevated, extra-hepatic utilization of these metabolites in ketotic states (Barnes *et al.*, 1938, 1940; Mirsky & Broh-Kahn, 1937; Dye & Chidsey, 1939; Shaw, 1942; but contrast Deuel *et al.*, 1940). The most important pathway known for ketone oxidation involves formation of acetyl coenzyme A, and subsequent metabolism *via* the tricarboxylic acid cycle. This would of course require oxaloacetate. It is therefore necessary to suppose that, in states of ketosis, the extra-hepatic tissues retain adequate amounts of oxaloacetate. If a deficiency does, in fact, arise it must then be limited to the liver. Such an organ difference is by no means impossible, but would appear rather unlikely. Moreover, it is to be emphasized that oxaloacetate is required in only catalytic amounts: the acid is regenerated during the operation of the tricarboxylic acid cycle. Admittedly, some degree of wastage is inevitable by both spontaneous and enzymic decarboxylation, but reactions by which oxaloacetate can be regenerated are also well known.

Carbohydrate may be metabolized to the pyruvate level, after which it is converted either to acetyl coenzyme A or to oxaloacetate (Fig.4). Of the pathways shown for oxaloacetate formation from pyruvate it is probable that [(2) + (3)] is the most important. Reaction (4) is unlikely to be important. The subject has been thoroughly discussed by Krebs (1954, 1957). More recently Hiatt *et al.* (1958) have reported that while dicarboxylic acids (such as oxaloacetate) are readily formed from pyruvate in liver, this is a much less important route in muscle. It has been known for some time that the rate of oxaloacetate synthesis is very rapid in liver. Thus, in addition to carbohydrate, it may be formed from the glycerol moiety of fats (metabolism of which should be increased *pari passu* when fatty acid catabolism is increased). Moreover, the glucogenic amino-acids (*e.g.* aspartate, glutamate, alanine) are also capable of giving rise to oxaloacetate (and again it is known that amino-acid catabolism tends to be increased in ketosis). These observations do not conform very well with the idea that there is a preferential loss of oxaloacetate from the liver in ketosis. It is, however, possible to argue that although oxaloacetate synthesis is very rapid in liver, losses may be even greater.

Thus, it is possible that, in states of ketosis, oxaloacetate is diverted to hexose synthesis, and that this is subsequently released into the blood to maintain the sugar level. Again, however, experimental results have been obtained which suggest that, although hepatic glyconeogenesis from oxaloacetate may well be increased in ketosis, enough of this dicarboxylic acid remains to support the operation of the tricarboxylic acid cycle. If ketosis is attributable to hepatic oxaloacetate deficiency, it would be anticipated that the severity of the ketosis should be correlated with the extent to which the cycle was suppressed. Medes et al. (1953) studied the conversion of isotopically labelled acetate to CO_2 and acetoacetate in rat liver slices. They found that, in slices taken from rats subjected to a short fast, the conversion of acetate to CO_2 was essentially normal. This suggests that, in these preparations, the Krebs cycle was operating normally. However there was a large increase in acetoacetate synthesis. Provided, therefore, that acetoacetate synthesis reflects total ketone synthesis, increased ketogenesis occurred when inferentially, oxaloacetate deficiency did not exist. It will be recognized that extremely large changes in the acetoacetate/beta-hydroxybutyrate production ratio might modify this conclusion. However since, in earlier experiments, this ratio was found to be approximately unity (Weinhouse, personal communication) the conclusion would appear to be valid.

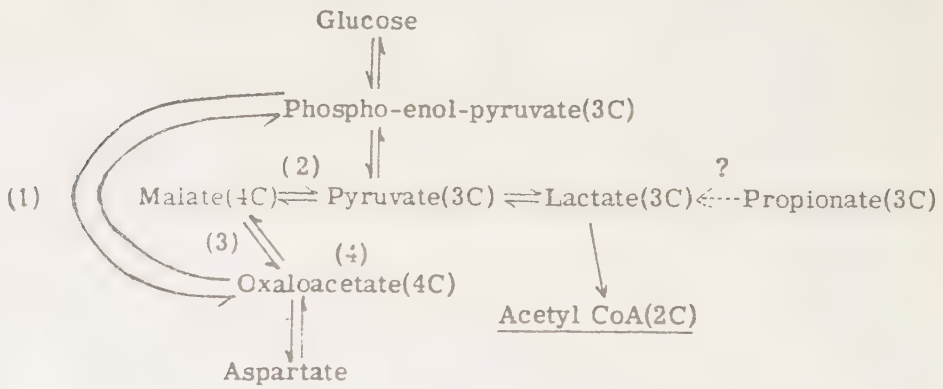

Figure 4.

Legend for Fig. 4.

(1) Utter & Kurahashi (1953).
(2) & (3) Korkes et al. (1950).
(4) Utter & Wood (1946).

More recently direct evidence has been obtained by studying tissue levels of oxaloacetate in fed, fasted and alloxan-diabetic rats (Kalnitsky & Tapley, 1958. Shaw & Tapley, 1958). It was found that liver from fed and fasted rats contained identical amounts of oxaloacetate. However "fasted" liver contained more than ten times as much acetoacetate as did

"fed" liver. Essentially similar results were obtained in alloxan-diabetic rats. It would thus seem that neither the ketosis of starvation nor of alloxan-diabetes can be due to hepatic oxaloacetate deficiency.

This is consistent with observations that oxaloacetate precursors fail to relieve ketosis (Lawrence et al., 1937; MacKay et al., 1938; MacKay, Barnes, Carne & Wick, 1940; Beatty & West, 1955; Fasella et al., 1958). Succinate has even been reported to increase ketonuria (Dibold et al., 1937).

It is of interest that Beatty et al. (1959) reported decreased extra-hepatic ketone utilization in alloxan-diabetes. Estimation of extra-hepatic oxaloacetate levels, in this condition, would probably be of great interest.

The apparent inadequacy of the oxaloacetate deficiency hypothesis makes consideration of other possible explanations necessary.

(b) The alternative metabolic pathway hypothesis. Brady & Gurin (1950) suggested that increased ketogenesis resulted if lipogenesis was suppressed. It was supposed that acetyl coenzyme A was diverted from fatty acid synthesis to ketone body synthesis. Evidence has accumulated that a number of conditions under which ketosis occurs are also marked by decreased lipogenesis.

Stadie (1958) and Siperstein (1958), in recent reviews, put forward this hypothesis in somewhat modified forms.

Again, unfortunately, objections seem to exist. Thus examination of the data of Medes et al., 1953 (Table 1), reveals that the quantitative relationship between lipogenesis suppression, and increased synthesis of acetoacetate, is very poor. Again, it would seem unlikely that changes in the acetoacetate/beta-hydroxybutyrate production ratio could be large enough to alter the conclusion appreciably.

Table 1.

Data of Medes et al. (1953).
Metabolism of radio-acetate by rat liver slices.

		Average total synthesis in micro(gram) atoms/g. dry wt./hr.	
Nutritional State	Specific activity of acetoacetate	Acetoacetate	Fatty Acids*
Fed	12130.0	63.9	33.8
Fasted, 12 hr.	12121.0	184.0	1.65
Fasted, 36–48 hr.	16949.0	90.0	0.53
Fasted, 72–120 hr.	9091.9	85.0	0.11

* Recalculated from the data of Medes et al.
(I am grateful to Dr. Weinhouse for kindly confirming these figures)

If a poor correlation, between decreased lipogenesis and increased ketogenesis, should be confirmed, then it would appear necessary to reject the hypothesis, at least in its original form.

It is possible to circumvent this objection by supposing that lipogenesis is suppressed in other tissues (e.g. adipose tissue), as well as in liver. This supposition would appear probable. It may then be supposed that the increased hepatic ketogenesis is correlated with the total body decrease in lipogenesis. Clearly this modified hypothesis requires the supposition of a more elaborate control mechanism, possibly humoral, than the original (which could be viewed as a mass-action hypothesis).

If this is correct, then it would seem more profitable to express the hypothesis in terms of the control mechanism, rather than the consequences of the control mechanism (see section on "Endocrine Balance" below).

(c) Deficiency of reduced triphosphopyridine nucleotide (TPNH) and/or decreased oxidation via the phosphogluconate oxidative pathway. It is probable that the phosphogluconate cycle (the "direct glucose oxidative cycle") is the major source of TPNH for animals. TPNH formation from other sources, e.g. iso-citrate oxidation, is quantitatively much less important. It has been suggested (de Duve & Hers, 1957), that the rate of phosphogluconate oxidation may depend on the demand for TPNH. Some in vitro work lends support to this view, thus TPNH acceptors, such as pyruvate, were found to increase direct oxidation of glucose (Kinoshita, 1957). It will also be apparent that the operation of the phosphogluconate pathway will be dependent on the availability of substrate, i.e., of carbohydrate.

Langdon (1957) has demonstrated a TPNH requirement for lipogenesis. Possibly, therefore, a decrease in phosphogluconate oxidation leading to reduced TPNH generation, and reduced lipogenesis, could lead to increased ketogenesis (see Stadie, 1958 and Siperstein, 1958 above). In the case of the cow, it seems possible to modify this argument somewhat. Perhaps, in mammary gland at peak lactation, there is a very high carbohydrate requirement: for lactose, glycerol and non-essential amino-acid synthesis, and for oxidative purposes to supply energy and TPNH. Conceivably, under these conditions, mammary gland (and perhaps certain other tissues such as the central nervous system) takes priority for the limited supplies of carbohydrate available to the ruminant. In consequence the liver may be deprived of carbohydrate, and increased hepatic lipide catabolism and ketogenesis may result. Control mechanisms for such a process are rather obscure. Possibly decreased hepatic phosphogluconate oxidation, and decreased TPNH generation, may play a part. In addition endocrine factors may be involved.

It would seem possible that, if carbohydrate deficiency becomes excessively severe, even the mammary gland may run short. If this occurred the observed fall in butter-fat in bovine ketosis might be the consequence of decreased TPNH generation.

Carbohydrate lack might also affect the adrenal cortex (where high phosphogluconate activities have been observed), perhaps resulting in decreased glucocorticoid production. This, by affecting gluconeogenesis,

might still further exacerbate the carbohydrate deficiency.

Moreover gluconeogenesis may be directly responsive to TPNH deficiency (Krebs, 1954).

(d) Extra-hepatic acetyl coenzyme A deficiency. Ketosis is possibly characterized in extra-hepatic tissues by a deficiency of acetyl coenzyme A. Suppression of phosphogluconate oxidation (by carbohydrate lack) might lead to increased demands for acetyl coenzyme A, for oxidative purposes, to replace the lost energy supply. Furthermore, an inadequate supply of acetyl coenzyme A (due to carbohydrate and/or acetate lack) might develop. Even if the supply of acetyl coenzyme A, from these sources, remained constant, a relative deficiency might result, in conditions of increased demand such as lactation.

To some extent an acetyl coenzyme A deficiency could be met by increased extra-hepatic metabolism of fatty acids. It is well established that many tissues contain enzyme systems for fatty acid catabolism (Lehninger, 1946a, b; Grafflin & Green, 1948; Geyer et al., 1949; Kennedy & Lehninger, 1950). The blood non-esterified fatty acids (NEFA) are believed to be of major importance in fatty acid transport to the tissues (Fredrickson & Gordon, 1958; Lindsay, 1959). However, circumstantial evidence suggests that, under conditions of severe energy deficit, triglyceride mobilization may also be required. The liver seems to play a predominant role in triglyceride catabolism (Geyer et al., 1951). Possibly then, situations can arise in which the extra-hepatic tissues cannot meet their full requirements for acetyl coenzyme A from dietary acetate and carbohydrate, and from mobilized NEFA. It may then be that increased hepatic lipide catabolism becomes important, and that this, through increased hepatic ketogenesis, leads to increased release of ketone bodies to the blood. These ketones could then serve as substrates for extra-hepatic metabolism, so helping to fill the postulated acetyl coenzyme A deficit in these tissues. From this somewhat teleological viewpoint, ketosis would be related to hepatic overproduction of acetyl coenzyme A, leading to increased hepatic ketogenesis, both effects being consequent upon an extra-hepatic acetyl coenzyme A deficit (cf. Weinhouse, 1955; Krebs, 1959).

Control mechanisms for such a process are again obscure, but are possibly humoral. Important features would doubtless be control of mobilization of NEFA and triglycerides from adipose tissue, and transport of fat to the liver. Direct effects on hepatic fat catabolism and ketogenesis are also possible.

Shaw & Tapley (1958) have also speculated that over-production of acetyl coenzyme A, in liver, may be responsible for the development of ketosis. The mechanism suggested by them has the merit of greater simplicity, however, the writer feels that extra-hepatic control mechanisms are probably involved. A possible mechanism for regulation is discussed in the next section.

(e) Endocrine balance. Dye & Chidsey (1939) suggested that ketosis may arise in consequence of changes in endocrine balance. Hormonal effects on ketone metabolism are well known and this concept would appear to have much to recommend it, particularly since other suggested mechanisms for the development of ketosis are by no means firmly

established.

It seems clear that diet is related to the development of ketosis in some way. Further, it is known that, in at least some ketotic states, the metabolism of carbohydrate is suppressed. Since ketosis is generally regarded as a consequence of increased hepatic ketogenesis, it is reasonable to look for an effect of carbohydrate on lipide metabolism and ketone synthesis by the liver. Such an effect is in fact demonstrable. Geyer & Waddell (1951) found that, while fasting increased fatty acid oxidation, prior administration of carbohydrate produced a decrease. Later Geyer et al. (1953) found that the addition of pyruvate suppressed in vitro ketogenesis by liver slices taken from fasted rats. Generally similar results were obtained by Beatty et al. (1958) with succinate, fumarate and oxaloacetate. In contrast, Allen et al. (1955) found that in vitro addition of glucose to liver slices did not affect the metabolism of fatty acids. This discrepancy appears to be resolved by the experiments of Lossow et al. (1956). They found that while the in vivo administration of glucose suppressed fatty acid catabolism and ketogenesis, addition of glucose in vitro was ineffective. Fructose was effective in vitro, but the magnitude of the response was less than with glucose pre-feeding. While other explanations possibly exist, these results would seem consistent with an effect of carbohydrate on hepatic ketogenesis mediated by some other organ or organs. One such mechanism might be an effect of carbohydrate on the secretion rate of certain hormones. The concept that some endocrine secretion rates may be controlled by the composition of the metabolic mixture, made available from the diet, has certain very attractive features.

Interactions between the diet and the endocrine glands have been discussed in a very stimulating book by Samuels (1948). Elevated plasma glucose levels have been reported to produce increased insulin secretion by the pancreas (Anderson & Long, 1947; Foà et al., 1952; Wrenshall et al., 1955). It seems probable that the anterior pituitary is prominently involved in the control of metabolism. Direct effects of carbohydrate availability on pituitary function seem rather unlikely. However it is known that at least some pituitary secretion rates are controlled by hypothalamic centres (Donovan & Harris, 1957). According to current theory, central nervous tissue should be highly dependent upon carbohydrate metabolism, and might well be responsive to changes in carbohydrate availability. It is of interest that the presence of "gluco-receptors" has been postulated in the hypothalamic area (Mayer, 1953; Forssberg & Larsson, 1954).

It would not seem profitable, at this time, to enter into a detailed discussion of ways in which endocrine control of metabolism might be responsive to changes in the composition of the metabolic mixture, specifically perhaps, to carbohydrate availability. However, in general terms, it might be supposed that the secretion rates of two (sets of) hormones, affecting carbohydrate and fat metabolism respectively, are linked in reciprocal fashion, or alternatively, that the secretion of one or more hormones affecting both carbohydrate and fat metabolism is controlled by the composition of the metabolic mixture.

It is necessary to admit however, that other non-endocrine control

mechanisms, linked to carbohydrate availability, may be involved. Very important in this connexion may be an effect of carbohydrate on release of NEFA from the fat depots. The addition, *in vitro*, of glucose has been found to inhibit NEFA release from adipose tissue (Gordon & Cherkes, 1958). In contrast, Goodman & Knobil (1959) found that, *in vivo*, adrenaline produced mobilization of NEFA only in animals with intact pituitaries. Lindsay (1959) has reported that, in sheep, acetate is as effective as glucose in inhibiting release of NEFA. Observations by Feller & Feist (1957) that, in the rat, propionate may be prominently involved in adipose tissue lipogenesis, seem of considerable interest. Possibly a deficiency of propionate could lead to decreased adipose lipogenesis and increased lipolysis.

BOVINE POST-PARTURIENT KETOSIS

Ketosis is of very widespread occurrence in cattle. It occurs as a complication to many diseases in early lactation, and cases have even been reported (Kingrey *et al.*, 1957) in cows in late pregnancy. The condition is not limited to the dairy cow, cases have also been reported in beef cattle (Hendershot, 1946).

The following account will be limited to the condition as seen in lactating dairy cows, so-called "primary" ketosis (Shaw, 1955), where it would seem that ketosis, or more exactly the metabolic disturbance accompanied by ketosis, is the most important feature.

Incidence

The condition seems to occur in dairy herds all over the world. It is characteristically a disease of early lactation. Probably most cases occur two to six weeks post-partum, but cases have been reported up to about three months after calving (Sjollema & van der Zande, 1923; Allcroft, 1947; Shaw *et al.*, 1953).

A marked seasonal influence on the number of cases appearing is well known. In Gt. Britain most cases occur in winter and early spring. It was found by Eden & Green (1940) that blood and urine ketone levels were highest in February and March, while cows were stabled, and lowest in summer at pasture. Clinical cases were not observed in this experiment, however. The question of the influence of time of year is complicated by seasonal calving, by the antiketogenic effect of grazing (Sjollema & van der Zande, 1923), and by the ketogenic effect of certain winter feeds (*e.g.* Penny, 1958). It is of interest that cut grass has been alleged to be less effective in preventing the disease than is pasture (Sjollema, 1931-32; Holmes, 1950). Some have attributed this to a beneficial effect of exercise. However, it should be noted that in Jersey, where cattle remain at pasture throughout the year, cases occur at all times (Allcroft, 1947).

The disease occurs in many breeds (Fincher, 1936; Kingman *et al.*, 1945) and predominantly in heavy milkers. There is some evidence that genetical factors are involved (Harrison, 1951).

Correlation with age is poor (Hayes, 1931; Allcroft, 1947; Messervy, 1952; Nichols, 1952).

The disease is rarely fatal, but the decrease in milk yield leads to serious economic loss. Shaw (1955) estimated that, in the U.S.A., this amounts to as much as ten million dollars per annum, and that about a million cases develop yearly. In some cases milk yield may drop by 50%. Moreover, there is often a serious loss of condition and body weight may fall by as much as 200 lb. (Durrell, 1943). Evidence also exists that subclinical ketosis is widespread, and may lead to important losses of milk production.

Blood and urine chemistry, and blood volume

Sjollema & van der Zande (1923) reported high blood and urine levels of the ketone bodies in cases of the disease. This has been subsequently confirmed by many workers. Sjollema at first found the blood sugar level was normal, however, the method used for the determination was of very poor specificity. Later (1927) he found the blood sugar to be low.

Stinson (1929) also reported hypoglycaemia in clinical cases, and this has been frequently confirmed, however, normal blood sugar levels have also been encountered in some cases (e.g. Boddie, 1935).

Carlström (1950) reported that plasma potassium was rather low in the disease but this has not been confirmed. Saarinen & Shaw (1950a), and Seekles & Talsma (1951-52) found normal levels. Plasma levels of calcium, magnesium, sodium, inorganic phosphorus, and chloride have been reported to be essentially normal (Duncan et al., 1939; Saarinen & Shaw, 1950a). In contrast Halse & Velle (1958) found that serum calcium was depressed when blood ketones exceeded 45 mg. per 100 ml. In three cases clinical recovery, and improvement in appetite, was associated with return to normal of serum calcium.

Decreased serum phosphatase has been found (Duncan et al., 1939; Shaw et al., 1949). This was believed to be due to the poor nutritional state of the animals.

Puntriano (1952) found the urinary excretion of glucocorticoids to be decreased in bovine ketosis, compared with normal lactation. The results were interpreted as indicating decreased adrenocortical activity in ketosis.

Robertson et al. (1957a, b) reported low values for plasma protein-bound iodine in ketotic compared with normal lactating cows. The plasma free 17-hydroxycorticosteroids were found to be moderately elevated.

Bach & Hibbitt (1959) found that serum pyruvate and alpha-oxoglutarate levels were elevated, while serum citrate and succinate levels were depressed in bovine ketosis. These sera were obtained from fluorided blood samples 12 to 24 hours after collection. Evidence has been obtained for large in vitro changes in pyruvate concentration in fluorided blood (Pearce, unpublished observations). Further, it would seem possible that the low citrate levels found may be due to inanition. Low levels have been observed in cases of Johne's disease. Bach & Hibbitt infer that the blood levels found possibly reflect the levels in tissues, and believe that their results are consistent with decreased tricarboxylic acid cycle activity. It should be pointed out that reduced levels of tricarboxylic acid cycle intermediaries need not necessarily

mean decreased cycle activity (see for example Katz & Chaikoff, 1955a, b).

Craige (1950) studied the free fatty acids in bovine blood. He found that, in normal animals, these rose before parturition to a maximum three days post-partum. Subsequently the level fell. However, in cows which later developed ketosis, the level remained high until the onset of symptoms.

Saarinen & Shaw (1950a, b) found that, while blood lipide fractions in ketotic cows differed from normal, similar changes occurred in underfed cows, which did not develop clinical signs despite the presence of ketosis, and hypoglycaemia.

Observations of this sort suggest that neither ketosis, nor hypoglycaemia, can fully account for the development of the clinical condition (see also Eden & Green, 1940; Shaw & Leffel, 1949).

Similarly acidosis does not appear to be responsible, although it may be a complication in protracted cases (Sjollema, 1931–32; Sampson & Hayden, 1935; Sykes et al., 1941).

Adler et al. (1955) studied the effect of injecting iso-propanol into normal cows and concluded that this ketone body might be responsible for certain of the signs seen in the "nervous" type of case. Robertson & Thin (1953) found this ketone to be higher in clinical cases than in fasted cows without clinical signs.

Vigue (1955) reported high haematocrit values, and low blood volumes, in ketotic cows, but opposite results were obtained by Rouertson et al. (1956).

Metabolic changes

Robertson et al. (1956) found reduced bromsulphalein clearance in ketotic cows, suggesting decreased liver function. Clearances were improved with improvement in the clinical state.

In liver homogenates, from two cases of ketosis, Sauer et al. (1958) found a marked decrease in respiration rate.

Peeters et al. (1958) reported decreased acetylation of sulphanilamide in ketotic cows. This was improved by glucose administration, and restored to normal by glucose plus insulin.

Bouckaert et al. (1958a, b) reported that injected acetate was utilized more slowly in ketotic than in normal cows. In contrast, fasted, normal cows showed an improved "acetate tolerance". This latter finding contrasts with that of Reid (1958) for the fasted sheep. Bouckaert et al. also reported that both normal and ketotic cows responded to acetate administration with a similar rise in blood ketones.

Chung et al. (1959), using an isotopic technique, studied liver metabolism of volatile fatty acids in ketosis. A marked decrease in formate utilization was found, and the pattern of utilization of the other volatile fatty acids was altered. Certain of the results were taken to be consistent with decreased gluconeogenesis from amino-acids in ketosis.

Aetiology

The aetiology of the condition remains obscure. There seems to be little doubt that an important factor is the heavy demand for carbohydrate by the lactating mammary gland which may amount to more than

1 kg. per day in heavy milkers (Shaw et al., 1942a). It would appear, however, that some other factor is probably involved. Among the various suggested causes are:

1. A dietary deficiency (Hoflund & Hedström, 1948; Hoflund, 1952; Breirem et al., 1954; Vigue, 1958a).

(a) A deficient energy intake.
(b) A deficient carbohydrate intake.
(c) An imbalanced carbohydrate intake (cellulose vs. soluble carbohydrate).
(d) A high protein (i.e., low carbohydrate ?) diet.
(e) A low protein diet.
(f) A mineral deficiency (e.g. calcium, phosphate, cobalt, manganese, or copper.
(g) A vitamin deficiency (e.g. vitamin A or thiamine).
(h) Excessive silage feeding giving either an excessive intake of butyrate (Brouwer & Dijkstra, 1938; Knodt et al., 1942; Todd, 1958) or a high intake of ketone bodies or acetaldehyde (Adler et al., 1958).

2. Ruminal dysfunction – possibly consequent upon faulty nutrition or a sudden change in ration (Hoflund & Hedström, 1948, Clark & Weiss, 1955).

(a) A decreased production of volatile fatty acids, or an abnormal production with decreased propionate or increased butyrate (and acetate) production (see also El-Shazly, 1952; Tyznik & Allen, 1951; Card & Schultz, 1952; Elliot et al., 1957; Balch & Rowland, 1957).
(b) Abnormal production of ketone bodies in the rumen.
(c) Decreased ruminal synthesis of members of the vitamin B complex.

3. Endocrine imbalance (Shaw, 1955, 1956; Fincher & Hayden, 1940; Puntriano, 1952; Messervy, 1952; Robertson et al., 1957a, b).

(a) Pituitary-Adrenal cortex system.
(b) Pituitary.
(c) Thyroid-Adrenal cortex (with the possibility of pituitary involvement).

4. Hepatic dysfunction (Sjollema, 1931-32; Holmes, 1950; Robertson et al., 1956).

5. Lack of exercise (McEwen & Foggie, 1950).

6. Retention of uterine fluids (Hudson, 1928).

According to Shaw (1943) a deficient energy intake is unlikely to be the cause since he found that two cows showed ketosis while in caloric balance. Moreover, low energy intakes post-partum were found to affect blood sugar and ketones, but not to give rise to clinical signs (Shaw, 1946b; Shaw & Leffel, 1949). Such observations suggest that dietary deficiency cannot be the sole factor involved in the development of the condition.

The concept that ketosis arises as a result of ruminal dysfunction, perhaps occasioned by a dietary disturbance, has many attractive features. Again, however, agreement is not general. Thus, Brown & Shaw (1957) reported results which cast doubt on this idea. Comparison of ruminal volatile fatty acids failed to show a difference between healthy, fasted cows and cases of clinical ketosis which were off their feed, or between fed, normal cows and clinical cases with good appetites.

While a mineral deficiency may sometimes be responsible, it

appears unlikely to be the cause, in a great many cases (Baltzer et al., 1941; Breirem et al., 1954). However, it is possible that a mineral supplement, such as cobalt, may sometimes produce a beneficial effect by stimulating appetite, even in the absence of a mineral deficiency.

Silage feeding, while possibly sometimes a precipitating factor, cannot account for a large number of cases.

It would appear that further work on possible endocrine changes in ketosis should be very profitable. Interactions between the diet and the endocrine glands seem worthy of consideration. The position is likely to be complex, and difficult to understand. Thus, if hepatic dysfunction occurs in ketosis, it might well affect the metabolism of both adrenocortical and thyroid hormones (Vannotti & Béraud, 1959; Englert et al., 1957; Urquhart et al., 1959; Brown et al., 1954, Klein et al., 1955). Moreover it has been reported that hypothyroidism results in a decrease in liver function, as judged by bromsulphalein clearance (Aterman & Howell, 1959).

Pregnancy and oestrogens have been found to affect certain parameters of thyroid function (Heinemann et al., 1948; Man et al., 1951, Feldman, 1956; Florsheim, 1958; Engbring & Engstrom, 1959). The pituitary may be involved in these effects (Badawi & Soliman, 1957). It has been suggested that normal pregnancy is followed by relative hypothyroidism (Danowski et al., 1953).

Armstrong & Hansel (1956) found that, in heifers, the pituitary content of thyrotrophic hormone correlated with the level of nutrition. Higher pituitary levels were found on the higher levels of nutrition, and it was believed that this was accompanied by increased secretion of thyrotrophin. High protein diets have been found to influence the thyroid (Greaves et al., 1959).

Sørensen (1958) found that, in cattle, the serum protein-bound iodine level rose in winter and fell in summer. Serum protein-bound iodine was also increased by pregnancy, but there was no evidence for an increased thyroid secretion rate. The iodine content of the thyroid was highest in summer and autumn, and low in spring. The findings for protein-bound iodine have been confirmed by Asplund et al. (1959), who also observed a correlation with silage feeding.

There is evidence from a number of species that the thyroidal secretion rate is increased in lactation (Rugh, 1951; Henneman et al., 1955; Flamboe & Reineke, 1957; Grosvenor & Turner, 1958). This has also been found in the cow by Sørensen (1958), who observed that the secretion rate was proportional to the butter-fat yield.

It seems possible therefore that pregnancy, high levels of nutrition, season, and lactation all exert a marked influence on the metabolism of the thyroid, and that bovine post-parturient ketosis may be due to a failure of thyroid function. In this connexion the observation of very low plasma protein-bound iodine in clinical cases (Robertson et al., 1957a, b) seems of extreme interest. These workers also suggest that hypothyroidism leads to a relative adrenocortical deficiency. This would accord well with the observations of others (Evans et al., 1939; Levin & Daughaday, 1955; Peterson et al., 1955; Peterson & Wyngaarden, 1956; Eik-Nes & Brizzee, 1956; Brown et al., 1958). There is evidence that

one effect of hypothyroidism may be to decrease adrenocortical hormone turnover, presumably in the peripheral tissues. However an effect mediated through the pituitary seems possible also. Zarrow & Zarrow (1951) suggested that, if secretion of thyrotrophin was increased, there might result decreased secretion of adrenocorticotrophin. It is of interest that a possible reciprocal relationship may also exist between pituitary secretion of thyrotrophin and of growth hormone (Contopoulos et al., 1958).

As a still further complication, effects of the adrenal cortex on the thyroid have been reported (Bastenie & Ermans, 1958; Brown-Grant et al., 1954; Money, 1955). Some of these may well be exerted at the peripheral level, but effects mediated by the pituitary are also possible (Knigge, 1957).

Shaw and his co-workers, in a series of papers (Hatziolos & Shaw, 1950; Shaw et al., 1950; and see Shaw, 1955, 1956), present their view that clinical ketosis in the bovine is due to adrenocortical insufficiency, possibly consequent upon pituitary exhaustion. Shaw (1955) believes that the condition arises when unknown stresses are superimposed upon the normal stresses of pregnancy, parturition and lactation.

Treatment

Since there is no general agreement as to the fundamental cause(s) of ketosis, the best form of treatment remains uncertain. There have been violent fluctuations of opinion in the past, and distinct vogues in treatment are discernible. At present there is a tendency towards increased use of corticosteroids, particularly the more potent synthetic products. This treatment has, perhaps, rather more justification than some which have been popular in the past (and, indeed, often continue in use). However, the theoretical justification, and therapeutic benefit, of corticosteroid therapy is by no means unimpeachably established.

(a) Carbohydrate, glucogenic substances and volatile fatty acids. One of the oldest treatments, which is still popular, is the administration of some form of soluble carbohydrate (Stinson, 1925; Hudson, 1931; Boddie 1935; Sampson & Bowley, 1941; Shaw & Powell, 1941). There has been an increasing tendency for this to be administered by vein, or subcutaneously, rather than by mouth. Oral administration has the disadvantage of possible digestive disturbances, and moreover, the administered carbohydrate is extensively altered as a result of ruminal fermentation. Subcutaneous administration can lead to undesirable reactions which Johnson (1951a) found could be minimized by including hyaluronidase in the glucose injection.

Many cases are glucose resistant (McConnell, 1940; MacKay, 1943; Shaw, 1955), for these Roberts & Dye (1951) proposed continuous intravenous glucose infusion. The technical difficulties involved will be apparent. Sampson (1947) believed carbohydrate therapy was specific. Johnson (1951b) found large doses of glucose by vein to be very effective, provided the treatment was sufficiently extended. O'Rourke (1953) claimed that 90% of cases responded to intravenous glucose plus chloral hydrate.

In a rather different approach glucogenic substances, which are less

affected by ruminal micro-organisms than is glucose, have been administered by mouth. Propylene glycol (1,2-propanediol), in doses between 200 and 800 g. per day has been used with success (Johnson, 1951b; Mills, 1954; Maplesden, 1954; van der Kooi, 1958). It is probable that this compound is metabolized via 1,2-propanediol-1-phosphate to a three-carbon glycolytic intermediary. Since Rudney (1954) has suggested that the ketone bodies may be metabolized to carbohydrate through this intermediary, the treatment may not be ideal. Conceivably endogenous gluconeogenesis from ketones might be depressed by propylene glycol administration.

Glycerol, per os, has been used with apparent success (Johnson, 1951b).

Ammonium lactate has been used by Seekles (1951), others have preferred a mixture of sodium and calcium lactate (Shaw et al., 1955a; Worden & Reid, 1958). Shaw et al. found this to be palatable and very effective.

Sodium propionate by drench, capsule, or mixed with the food, has been found effective (Schultz & Smith, 1951; Schultz, 1953; Shaw et al., 1952a, but contrast Shaw et al., 1953). Vigue (1955) found this salt led to digestive troubles.

Sodium acetate administration may be beneficial (Tyznik & Allen, 1951; Miller & Allen, 1952a; but contrast Shaw et al., 1952). Miller et al. (1954) wrote "The frequency of recovery was sufficiently high to indicate either that the acetate treatment was beneficial, or that there may be reason to question the justification for routine treatment". Piper (1952) also obtained good results with acetate and with acetate plus glucose. The combined treatment was found to be superior to glucose alone.

(b) Vitamins, amino-acids, ruminal juice and strychnine. Success has been claimed with thiamine treatment by Carlström et al. (1941), Steyn (1941) and Haney (1946). This has been disputed by Forbes (1943), Shaw (1946a), and Bouckaert et al. (1947).

Vitamin A was found to be very effective by a number of workers (MacKay, 1943; Patton, 1944, 1945; Burt, 1944; Horner, 1945; Messmore 1945). It has also been used in conjunction with glucose or brown sugar (Sisk, 1945; Scott, 1945). Shaw et al. (1945) failed to find hypovitaminosis A in cases of ketosis, and did not obtain a favourable response with the vitamin. Hayden et al. (1946) also failed to cure the condition with vitamin A. Shaw (1950) fed diets low in carotene for nearly five months without producing ketosis. In contrast, Falster (1948) found low serum vitamin A in 11 cases of ketosis. These, and additional cases, were successfully treated with vitamin A.

Fohlin (1947) claimed a favourable response to massive doses of vitamin D.

Vitamin C has been claimed to give a favourable response in some cases (Stilinović, 1957; Bouckaert et al., 1958a).

White (1955) found benefit from intravenous vitamin B_{12}.

Talsma (1952) claimed choline chloride intravenously improved some cases.

Lecomte (1954) reported methionine effective, but Shaw (1946a) obtained a negative response with this amino-acid.

Spörri (1958) has suggested that, after initial treatment (with carbohydrate, corticosteroid, or chloral hydrate), it may be helpful to inoculate with fresh rumen juice.

Mills (1954) suggests, among other treatments, the use of a strychnine tonic.

(c) Inorganic substances. Cobalt was found to be effective by Ender (1946), Henderson (1947), Hallgren & Sandstedt (1947), Hunter et al. (1950), and White (1955). Cobalt deficiency was not always established, and it was considered in some cases, that an effect on appetite might be involved. Breirem et al. (1949, 1954) failed to prevent the development of ketosis with cobalt (or with phosphate or copper).

Burrow & Lewis (1950) reported success with magnesium sulphate subcutaneously. Hypomagnesaemia was not present, but it was suggested that possibly the plasma magnesium might be in a non-ionic form.

Sjollema (1951) has suggested that potassium chloride might be beneficial. Talsma (1952) failed to confirm this, however success was claimed with potassium (or sodium) chlorate. Similar results have been obtained by Seekles (1951) and Good (1958).

Continued treatment with dilute potassium permanganate was found effective by Cardassis (1956).

Sodium bicarbonate has often been recommended (Sjollema, 1931-32; Boddie, 1935; Skamser & Proewig, 1958), to treat a suspected acidosis (which is not, however, invariably present). It has generally been combined with some form of carbohydrate therapy.

Chloral hydrate has been very extensively used, often in conjunction with glucose. It is said to be contra-indicated in milk fever complicated by ketosis (Fincher, 1936), or if the animal is weak or emaciated (Sampson, 1947).

Skamser & Proewig (1958) have suggested that sodium fluoride should be administered to cases of bovine ketosis.

(d) Hormones. Insulin, combined with carbohydrate, treatment has been suggested (Sjollema, 1931–32); Bouckaert et al. (1958a) found this no better than carbohydrate alone.

Thyroprotein (Reece, 1947), and thyroxine (Vigue, 1958a), have been reported to be beneficial.

Stilinović (1958) has used stilboestrol, in combination with various other treatments (vitamin C, saline, calcium carbonate and yeast).

Anterior pituitary extracts have been administered by a number of workers (Fincher & Hayden, 1940; Gibbons, 1940; Dennis, 1941). Additionally anterior pituitary adrenocorticotrophin has been claimed to cure the condition (Shaw et al., 1953; McAuliff et al., 1954; Rogers, 1954). Talsma (1954) found this to be no better than other treatments.

Adrenocortical extract was found beneficial by Shaw (1947). At this time he considered that an adrenocortical deficiency was unlikely. However, he later adopted this idea partly because of histological changes encountered in adrenals and pituitaries from cases of bovine ketosis, and partly because of continued success with corticosteroid therapy. Success has been claimed with cortisone treatment by Shaw et al. (1950, Shaw et al. (1953), Dye et al. (1953), Bowlby & Comfort (1953) and Shaw et al. (1955b). Hydrocortisone was used by Shaw et al. (1953) and Gessert et al.

(1955). The required dosage was rather high for both cortisone and hydrocortisone. Using 9-alpha-fluoro hydrocortisone the effective dose was reduced to about 100 mg. (Shaw et al., 1955a).

Prednisolone has been found effective by Paterson (1957), Link et al. (1957), Rogers (1958), Huber (1958), Vigue (1958b), and Bouckaert et al. (1958a).

The evaluation of a method of treatment is notoriously complicated by spontaneous recoveries (Shaw, 1946a; Miller et al., 1954), and it would seem possible that at least some claims of success are due to this effect.

Prevention

Various attempts have been made, mainly by dietary means, to prevent clinical bovine ketosis.

Feeding soluble carbohydrate has been found ineffective as a preventive measure (Shaw, 1943; Messervy, 1952; Fincher, 1950). Breirem et al. (1949) considered that diets rich in cellulose, but low in sugars, reduced the incidence of the disease. The level of energy intake pre-partum has been found not to affect the number of cases appearing (Schmidt & Schultz, 1959; Hathaway et al., 1957; Blackburn et al., 1958). Leffel & Shaw (1957) found that the level of protein fed pre-partum did not affect the development of fasting ketosis post-partum — clinical cases were not seen in this study.

Schultz (1953, 1958) found that sodium propionate (4 oz./day per os), for six weeks post partum, was effective in preventing clinical ketosis. The work of Richards (1958), feeding 2 oz. per day, may be contrasted.

Umberger & Bo ey (1956) and Derrer (1960) found that "Hi-amine" (ethylene-diamine dihydroiodide), added to the ration, was effective in prevention. They attributed this to thyroid stimulation, with subsequent effects on the pituitary and adrenal cortex.

Rogers (1956) claimed that intramuscular injection of an aqueous suspension of hydrocortisone acetate prevented the development of cases.

Conclusion

While the metabolic pathways for ketone body metabolism are now fairly well mapped-out, there is still uncertainty as to the control and integration mechanisms involved. In the special case of clinical bovine ketosis, it is not even clear how important a part ketosis plays in the development of the condition. Moreover there is little agreement as to the basic cause(s) of the disease, or as to the best methods of prevention and treatment. Interactions between the diet and the endocrine glands are suggested as a possible mechanism for the development of the disease. Further study of preventive measures, particularly those involving a dietary or hormonal approach, would seem to hold out some promise of success in reducing the incidence of the disease.

Note: The uncertainty still existing in any discussion of bovine ketosis is well illustrated by the recent claim (McCarthy, R.D. & Shaw, J.C., 1960. J. Dairy Sci. 43, 1010) that there is a decreased production of ketones by the liver in this condition. These workers suggest that extra-hepatic overproduction of ketones may account for the elevated blood levels.

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Can Swine Erysipelas be Eradicated? Epidemiological and immunological aspects

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Swine erysipelas is world-wide in its distribution. It occurs in all parts of Britain, but the incidence is higher and the infection usually more virulent in certain areas, e.g. Eastern Counties. It occurs throughout the year, but there is a great increase in the number and severity of outbreaks during the summer. It usually affects pigs from three months of age upwards. In America sucking pigs are frequently affected.

Although losses in Gt. Britain are not spectacular, the disease is a considerable burden on the industry, but not being notifiable, its full extent is unknown. Nevertheless the annual financial loss must be heavy and, in the light of existing knowledge, unnecessary. Losses result from deaths among fat pigs, loss of condition, unthriftiness, stunting and arthritis. Losses from condemnation at bacon factories are relatively light as compared with experience in America. Anthony (1959) estimated condemnation figures at 0.005% for arthritis, at 0.01% for acute cases and about 0.03% for chronic cases.

In Europe, especially Central Europe, the disease is so serious that it would be almost impossible to rear pigs profitably without prophylactic vaccination and serum therapy (Gledhill, 1959). In America the acute form is widespread and the economic loss is second in importance only to swine fever.

Holm, Griffith & Beeson (1942) described a joint form which was not a sequela of either the acute or the urticarial form. The main symptom was lameness affecting the carpal, tarsal and digital joints. Carrier sows appeared to be as important as infected land in the spread of infection. Strict sanitation and the elimination of breeding stock with an agglutination titre of 1:400 or over reduced the infection to a minimum. Schoening & Creech (1936) regarded the agglutination test as a means of detecting herd infection rather than a test of individual animals. Breed (1942) recovered Erysipelothrix rhusiopathiae from a high percentage of affected joints, but also from apparently normal joints. Hagan & Brunner (1957) mentioned that in some parts of the American 'corn-belt' erysipelas is one of the major problems of the pig industry.

At a Conference (1954) in Sioux City it was reported that: "in a recent slaughter test of 118,000 hogs, nearly 2,000 hams were rejected because of arthritis, most of which was probably due to chronic erysipelas infection." Neher et al. (1958) found arthritis to be the principal cause of condemnation of pig carcasses in spite of widespread immunization against erysipelas. Connell et al. (1952) noted an increase in arthritis in recent years in Canada. In 1956 18,059 carcasses were

condemned in packing plants, of which 213 were for acute erysipelas and 7,849 for arthritis (Ann. Rep. Vet. Dir., Canada).

Predisposing causes. Little is known about the predisposing causes of erysipelas; circumstantial evidence points to hot humid weather, rapid changes in barometric pressure, age, fat condition, bad hygiene and faulty nutrition as contributory causes. It would appear that pigs must be carriers if they are to react as quickly as they do to such extraneous factors. Predisposing causes appear to upset the acquired host-parasite balance and allow the parasite to gain the upper hand.

It may be merely a coincidence that the most devastating outbreaks of the disease in Europe and America occur in areas in which serum-virus immunization against swine fever is practised. Doyle (1947) observed that a high percentage of pigs immunized against swine fever (crystal violet vaccine followed by virus) proved susceptible to intracerebral inoculation of either culture or infected tissues (brain, spleen, blood) and also to intratesticular and intravenous inoculation of culture. Swine fever susceptible pigs were, on the contrary, strongly resistant to intracerebral inoculation with similar inocula. This observation has been confirmed by Thomson & Gledhill (1953), who inoculated culture intramuscularly into 'prepared' pigs. They used this method for assessing the protective value of an adsorbed vaccine and found it satisfactory. Challenge by the application of culture to scarified skin (Fortner & Dinter, 1944) proved unsatisfactory. Bolton & Bolton (1943) reported a severe outbreak of erysipelas among pigs which 11 days previously had been inoculated with serum-virus against swine fever. Nineteen pigs died within the following 48 hours. The predisposing cause was considered to be lowered resistance during the reaction to the serum-virus inoculation. Whether this increased susceptibility to erysipelas occurs in the field among pigs treated with serum-virus is not known, but it may well be the case.

Serum-culture method of immunization. The serum-culture method of immunization against swine erysipelas (Lorenz, 1896) has been extensively practised in Europe and America and has given satisfactory results.

Shuman (1953) has shown that vaccination of baby pigs (2 to 5 days old) did not protect to market age, and was of only limited value for protecting them to weaning age. Weaned pigs (average age 56 days) were protected for five to seven months. Immunity was estimated by the skin scarification test. Baby pigs, the progeny of gilts vaccinated as adults before service, were immune to scarification infection up to the sixth week of age; some protection was present up to the eighth week. This method was suggested as a means of protection from birth to weaning age. Lawson, Walker & Crawley (1958) found the serum-culture method to be usually successful, but it produced carriers. Ray (1958) considered that it confers the most solid and longest protection of the products available, provided pigs are susceptible and the dose of serum small. In answer to a questionnaire sent by Ray to veterinary surgeons many favoured the

vaccination of sows 4–6 weeks before farrowing. This procedure reduced the amount of baby pig vaccination; it is based on the observation that pigs reared on erysipelas-immune mothers acquire passive immunity from the milk which persists to six weeks of age. If these passively immune pigs are inoculated with culture they derive little benefit from it. In spite of the general use of serum–culture in Switzerland, the incidence of the disease increased (Saxer, 1947). There are many objections to this method. It is relatively expensive because of the serum; it requires two injections: and the virulent culture is dangerous to the operator. But the most serious objections are that inoculated animals may excrete virulent organisms which contaminate the ground, food and water and in addition it creates healthy carriers. So if used regularly it establishes the disease in a herd. The case is somewhat similar to that of serum–virus for swine fever, which is an excellent method of immunization, but it is now recognized in America that it would be difficult to eradicate the disease so long as it is practised and many States prohibit it.

Inactivated vaccine. Most inactivated vaccines are based on the work of Traub (1947), who showed that the antigenic structure of the vaccine strain of E. rhusiopathiae was the most important factor. Certain strains when grown in a suitable medium form a "soluble protective substance". These strains are rare: out of 73 strains examined only three produced it. They can be either virulent or avirulent; they belong to one serological group, but not all strains of the group produce the protective substance. Gledhill (1952) confirmed Traub's work. He found only a small number of strains suitable for vaccine; certain strains which failed to grow in ordinary medium grew when serum or liver extract was added.

Traub's vaccine is prepared by adsorbing the protective substance on to alum and combining it with the formolized bacteria. This vaccine, and others based on the same procedure, have been extensively tested against artificial (skin scarification) and natural infection in Germany, Poland, Yugoslavia and Switzerland and have given satisfactory results. Adsorbed vaccine has none of the disadvantages of the serum–culture method. The establishment of protection is however slower and its duration shorter, about four months with one dose and about six months with two doses.

Jungk & Murdock (1957) combined an adsorbate vaccine, prepared according to Traub's technique, with an emulsifying agent, mannide mono-oleate (Arlacel A) and a light mineral oil. This vaccine conferred protection against skin scarification for at least eight months. It caused occasionally a persistent nodule at the site of injection which sometimes became purulent.

Living avirulent vaccine. This type of vaccine is gradually coming into favour. Kondo & Sugimura (1935) used an avirulent vaccine on 46,000 pigs in an erysipelas infected district and only two died within one year whereas more than 300 unvaccinated pigs died of the disease in the same district. Staub (1940) used an avirulent vaccine on a large scale in France, Algeria and Poland with excellent results

According to Virat & Staub (1953) the protective dose of an avirulent strain containing the soluble protective substance was 125 times smaller than the same cultures killed and containing aluminium gel. Wiidik & Ehlers (1953) found that one ml. of avirulent vaccine gave the same protection as five ml. of adsorbed vaccine against skin scarification for a period of three months. Lawson et al. (1958) tested an avirulent strain against both skin scarification and natural infection. It protected 80–90% of pigs, 8 to 12 weeks of age, for about six months. They claimed the vaccine reduced or even eliminated arthritis.

An avirulent vaccine prepared at Weybridge was used on over 5,000 pigs in infected herds and good results were reported. The vaccine was avirulent for mice in a dose of 0.5 ml. of broth culture subcutaneously and protected them for more than one year (Doyle, 1952). It is relatively easy to immunize mice against E. rhusiopathiae; but they provide only a rough estimate of the efficacy of vaccine for pigs, the immunization of which is more complicated. A number of other avirulent strains of E. rhusiopathiae have been reported as conferring good protection. There is no evidence that any have reverted to virulence after passage in pigs. The advantages of these strains are good protection, a relatively small dose and cheapness of production which is important if vaccination is to be carried out as often as is necessary.

Soil infection. The question of soil infection is of great importance in the epidemiology of erysipelas. It would appear, from the literature, to be generally accepted that E. rhusiopathiae can live for a long time in soil; some observers believe it can multiply there when conditions are favourable. If this is true the prospects of eradication would appear hopeless.

Munce (1935) and Breed (1942) believed the disease could persist for many years on farms. In Australia, which is free from swine fever, erysipelas is almost invariably sporadic and it seldom recurs on the same farm (Seddon, 1956). Van Es & McGrath (1942) believed that the organism lives as a saprophyte in soil or wherever nitrogenous substances are decomposing; and that under favourable conditions it can multiply in soil. This is surprising as the organism is fastidious in its growth requirements under laboratory conditions. If it is a soil saprophyte it would be reasonable to expect infection to occur commonly among gardeners and others who frequently handle soil, but no cases are reported from this source.

Aitken (1950) observed heavy mortality in herds one year, yet non-immunized pigs, brought on to the same premises the following year, remained healthy; which would suggest that ground infection was not an important factor.

There is little experimental evidence to support the theory of persistent soil infection. It is not questioned that E. rhusiopathiae can survive for a time outside the animal body as can other non-sporing pathogenic bacteria, and that pigs may contract the disease from infected ground, food and water. It is the repeated assertion, based on flimsy circumstantial evidence, that soil is the main source of infection and that the organism can persist there almost indefinitely, that is doubted.

E. rhusiopathiae does not appear to have been cultured from naturally infected soil. Hesse (1923) inoculated different types of soil with the organism; those rich in humus, and sandy and calcareous soils were found to be particularly suitable for survival. Soil pH was the decisive factor. In alkaline soils the organisms were still viable after 70 days. They survived only a few days in acid soil. Obreshkov (1956) failed to isolate E. rhusiopathiae from the soil of farms grazed by erysipelas infected pigs. Direct sunlight killed the organisms in 12 to 20 days. Rowsell (1958) inoculated large amounts of culture into sterilized and unsterilized soil and the organisms persisted for only a short time. Six inoculations extended the period of recovery to a maximum of 21 days, but failed to produce a permanent infection—and this applied also to soil from areas in which erysipelas was endemic. He considered the general belief that soil is a frequent source of E. rhusiopathiae infection is not universally true.

Sukhoretskii (1958) inoculated 11 different types of sterilized soil with E. rhusiopathiae and incubated them at 11° to 18°C. The organisms remained viable for 12 to 14 months, but their virulence was greatly reduced. Subcutaneous inoculation of soil suspensions failed to kill mice, but they were killed by intraperitoneal inoculation. In similar samples of unsterilized soil the organisms rapidly lost their virulence and failed to kill mice even by intraperitoneal inoculation. Similar results were said to have been obtained by other investigators working with non-sporing bacteria. He considered that soil can be regarded as a secondary source of infection only when it is continuously and directly contaminated by the excreta of clinically affected or carrier pigs, and that claims of multiplication of E. rhusiopathiae in soils rich in organic material, with the persistence of virulence and other properties, have no foundation.

Gurova (1959) mixed a culture of E. rhusiopathiae with unsterilized soil of the black-earth type (pH 7.0) at a concentration of 250 million organisms per g. of soil. The organisms survived under summer conditions for 21–30 days; under winter conditions for 4–5 months. Direct sunlight killed them in two to five hours.

The theory of persistent soil infection seems to be based on the recurrence of the disease on farms which had been left vacant for a time after an outbreak or to its frequent reappearance on certain farms. To claim that contaminated ground is the main source of infection is to ignore a well-proved and more fruitful source—the healthy carrier pig.

The carrier pig. The existence of the healthy carrier pig is well established. Many observers, Pitt (1907), Bramm (1937), Dale (1937), Van Es (1942), and Spears (1954) have shown the carrier rate among pigs to vary from 30 to 50%. Spencer (1943) attributed spread of the disease to community sales. Breed (1942) believed the disease was taken to the Eastern States of America by pigs moved there because of drought in the Middle West. Hutyra, Marek & Manniger (1946) considered healthy carriers to be as infectious as clinical cases and they could give rise to the apparently spontaneous outbreaks which appear in the absence of any definite source of contagion. Carriers are especially dangerous when, under the action of secondary influences, they

develop clinical disease and excrete virulent bacilli. As outbreaks frequently coincide with the introduction of apparently healthy pigs, the importance of carriers cannot be overestimated. Connell & Langford (1955) isolated E. rhusiopathiae from 115 tonsils and from 192 gall-bladders and intestines, of 659 healthy pigs. Hagan & Brunner (1957) considered that carrier pigs spread the infection. According to Shuman (1959) the evidence points to the carrier as a significant source of infection, but that the relationship has not been sufficiently demonstrated. Rowsell (1958) found that E. rhusiopathiae shed by the tonsils of carriers may remain viable for at least three hours in the stomach (pH 1.5) and after passing through the intestines infect the ground. Gledhill (1959) believed that subclinical infections are common, at least in areas where the disease is known, and this suggests the carrier as the principal reservoir of the infection.

In many herds the disease persists in one form or another and circumstantial evidence suggests it is kept going by adult carriers infecting young stock which in turn pass it to the next generation.

Carriers among other species. Inapparent natural infection occurs among domestic animals (horses, cattle, sheep) and wild birds, but there is probably little risk of pigs contracting the disease from these sources. Lameness in sheep caused by infection with E. rhusiopathiae, after dipping in derris or benzene hexachloride solutions has been reported from many countries, the dipping solutions having apparently been contaminated by organisms from carrier sheep (Harbour, Whitten & Allan, 1943). Lambs may be infected through the umbilicus or through castration or docking wounds (Marsh, 1953). Kondo & Sugimura (1935) isolated 43 strains of E. rhusiopathiae from the body surface of 90 sea-fish. These strains were exceptionally virulent for mice and pigeons, but the inoculation of young pigs gave inconstant results—which is not unusual even with virulent strains. Grenci (1943) isolated E. rhusiopathiae from two samples of fish-meal which had been manufactured by the 'continuous' heat process.

Hagan & Brunner (1957) considered that fish products used as protein supplements in animal foods, may be responsible for introducing infection. They stated that E. rhusiopathiae has been isolated repeatedly from commercial fish-meal, indicating that the material is sometimes insufficiently heated.

Murase et al. (1959) claimed that strains of E. rhusiopathiae isolated from the body surface of fish are serologically distinct from the pathogenic strains (Groups A and B) and are of little significance to the disease in pigs. They believed these strains, which are present only on dead fish, to be post-mortem contaminants.

It is known, however, that handling fish is a common source of infection in man. Klauder et al. (1926) reported 1,000 cases of erysipeloid among commercial fishermen on the New Jersey coast. Gilchrist (1904) saw 323 cases caused by crab-bites or from contact with crabs. Hudson et al. (1952) reported 22 outbreaks of E. rhusiopathiae infection in turkeys. In six outbreaks the birds were reared on wire and in two they were confined to porches. In no case could the source of infection be

determined, but ground infection appeared improbable in view of the methods of rearing. Fish-meal, mice or rats were considered as possible origins. The possibility of adult healthy carriers does not appear to have been examined.

Discussion. The prospect of eradicating erysipelas depends to some extent on the epidemiology of the disease, which varies from one country to another; the methods used for controlling it; and the system of farming practised. Eradication does not appear to have been attempted yet in any country, probably because of the widely held belief that E. rhusiopathiae can remain viable for many months in the soil. There is little evidence in support of that view. On the contrary a strong case can be made out for the healthy carrier pig as the principal source of infection. It carries the disease from farm to farm and from generation to generation. In view of American experience the question of fish-meal as a source of infection should be examined.

The first step towards eradication is to stop the use of virulent culture for immunization. It creates carriers and the inoculated pigs contaminate the ground by infective faeces and urine. Its continued use in a herd perpetuates the infection. Having regard to the incidence of the disease the only rational way eradication can be achieved is by wholesale vaccination combined with good hygiene. Experience has proved that this will reduce the number of infected pigs. There is no completely satisfactory method of protection, serum–culture is probably the most effective, but its inherent disadvantages far outweigh its usefulness. Other vaccines are now available that are safe and confer a reasonable degree of protection on recently weaned, susceptible pigs; the vaccination of carriers is unlikely to be of benefit. Either an adsorbed vaccine or a living, avirulent strain can be safely used. The avirulent vaccine is cheaper to produce, the dose is smaller and it probably gives a longer protection.. There is a tendency to overstate the duration of protection conferred by erysipelas vaccines; four to five months is about the maximum period from one dose. The vaccine of Jungk & Murdock (1957) appears to be an improved form of adsorbed vaccine. In Britain the disease is usually mild during the winter and spring, but pigs recovered from mild infection may become carriers. The recommendation to vaccinate breeding-stock once a year is tacitly attributing to vaccine longer protection than it generally confers. Breeding-stock should be vaccinated twice a year. One dose of vaccine after weaning (8–9 weeks of age) should be sufficient for pigs destined for slaughter. Conditions in Britain appear to be favourable for eradication as the disease is generally sporadic and serum–culture is now seldom used. When pigs are kept in sties and yards the disinfection of ground is readily accomplished and is not a problem. But when they are run continuously on the same pasture losses from erysipelas are bound to occur. This is however a bad system and should be changed. Infective ground can be cleansed only by leaving it vacant for a time or by ploughing it. Eradication should be comparatively easy in closed herds provided regular vaccination is carried out at correct intervals and good hygiene practised. But the high incidence of healthy carriers would make it difficult at present to

eradicate infection from herds which recruit from outside sources, unless of course vaccinated stock can be purchased. To continue as at present to vaccinate only a small part of the national herd, leaving the bulk of it unprotected and subject to annual loss cannot be justified with existing facilities. The argument for eradication is based solely on economic grounds, the annual saving in a herd of even a small number of adult pigs, usually in good condition, would be a considerable financial gain and in addition the mild cases characterized by unthriftiness and arthritis would be eliminated. It would be essential to have a supply of good vaccine (adsorbed or avirulent) available at a reasonable price and there should be a wide dissemination of information to pig-farmers. The industry should be informed of the nature of the disease, the losses, control measures to be employed and the financial and other advantages of eradication.

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Pasteurellosis (*Pasteurella multocida*)

A review stressing recent developments

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Introduction. Within recent years two fields of investigation have contributed greatly to the expansion of knowledge of Pasteurella multocida. They are the study of virus infections of animals, and investigations on the serological and antigenic nature of P. multocida. Studies have shown that certain diseases, thought to be due wholly to P. multocida, were frequently a primary virus infection with a superimposed pasteurella infection. An example of such a mixed infection is virus pneumonia or enzootic pneumonia of pigs.

The newer knowledge of the serological types of P. multocida has contributed greatly to our understanding of their pathogenic activity. We now know, for example, that acute pasteurellosis or haemorrhagic septicaemia of cattle is almost always caused by one serological type of P. multocida, and that acute fowl cholera is generally due to another serological type.

Parallel with the growth of knowledge in the spheres referred to above was the development of vaccines capable of satisfactorily preventing

the ravages of epizootic pasteurellosis or haemorrhagic septicaemia of cattle. The use of these types of vaccines can be expected to reduce losses in other manifestations of pasteurellosis in cattle, pigs and poultry.

Another development in recent years has been the gradual realization that human infections yielding P. multocida are by no means rare. As this species becomes better known to the bacteriologist in human medicine we can expect the number of isolations from human beings to increase.

This review will attempt to assimilate and place in proper perspective the newer facts that have emerged in relation to pasteurellosis over the last two decades. In its compilation unpublished observations and material in press have been drawn on extensively.

Nomenclature. Serological studies on P. multocida have shown that in general the zoological classification of this species is unsatisfactory in that the same serotype may be recovered from different animal species. It is only rarely that one still sees names like P. bubaliseptica and P. boviseptica.

It has been the custom over the years to refer to the various strains of P. multocida as the haemorrhagic septicaemia pasteurellae or pasteurella. Some writers have also placed P. haemolytica under this same label. Certainly P. haemolytica does not usually produce haemorrhagic septicaemia except in lambs (Stamp et al., 1955), and it is doubtful if many of the strains of P. multocida of canine and feline origin possess the capacity to produce a haemorrhagic septicaemia. The term haemorrhagic septicaemia should only be used when referring to the acute septicaemic pasteurellosis of cattle. Considerable confusion would arise if acute fowl cholera were referred to as haemorrhagic septicaemia although in severe cases a haemorrhagic septicaemia may be observed.

The name P. multocida suggested by Rosenbusch & Merchant (1939) has found general acceptance on the North and South American continents. It is being used with greater frequency in European countries and Asia. P. septica is used in Great Britain and in some countries of the British Commonwealth. Although both names are acceptable, because P. multocida is more widely employed it is hoped that in the interest of uniformity it will gain universal acceptance.

It has been suggested that the serological type of P. multocida associated with epizootic pasteurellosis or haemorrhagic septicaemia of cattle be given separate species status. This would be inadvisable at present for the reasons that this serological type can only with assurance be distinguished from other varieties by serological means and that it is occasionally recovered from acute disease in animals other than cattle. It might, however, be worthwhile considering the designation P. multocida variety bovis. Roberts (1947) found that this type was separate immunologically from the other serotypes which he established. However, later work with the agar-gel diffusion precipitin test has shown antigenic relationships linking the whole group.

Identification. The identification of strains of P. multocida occasionally

presents some difficulty. The criteria listed in Bergey's manual (Breed et al., 1957) are generally accepted. However, a number of workers have noted that a small percentage of typical strains ferment lactose in varying degrees (Smith, 1958; Bain, unpublished).

Hall et al. (1955) described an organism that resembles P. multocida and P. haemolytica. Strains of this organism were isolated from lesions of chronic fowl cholera. They suggest that this organism, which is serologically distinct from P. multocida and P. haemolytica, be called P. gallinarum. It would seem premature to give it species status in the Genus Pasteurella until it is more widely recognized.

A pasteurella-like organism was recovered by Jawetz (1950) from pneumonic lesions in mice. It was found in the mice of a number of colonies surveyed and as well in guinea-pigs, white rats, cotton rats and hamsters. Observations on its serological, pathogenic and biochemical characters set it apart from recognized species of Pasteurella. The main significance of this organism which Jawetz named Pasteurella pneumotropica would appear to be its potentiality for complicating experimental respiratory infections in mice (Jawetz & Baker, 1953).

Bergey's manual lists a number of less well known species in the Genus Pasteurella. They are P. septicaemiae, P. anatipestifer, P. pfaffi and P. novicida. These should, according to the manual, be readily distinguished from P. multocida.

Distribution. This species would appear to be present wherever mammals and fowls are found. The host range is virtually unlimited.

Like many potential bacterial pathogens P. multocida is frequently found in the upper digestive and respiratory tracts of normal animals. When it is present in the lungs of pigs and cattle it is probably a secondary invader in low-grade viral lesions (Carter, 1957d; Carter & Rowsell, 1958).

Smith (1955) examined the noses and tonsils of 111 dogs for P. multocida. He obtained positive cultures from 10% of the noses and 54% of the tonsils. Smith's review of the literature on the subject of carrier rates in various species indicates a high percentage of carriers in the cat, dog, pig and rat. Singh (1948) suggests that his figures of 7% for dead and 3.5% for living cattle and buffaloes are probably low. His cultures were obtained from nasal swabs. Cultures of P. multocida can be readily recovered from a large percentage of swabs taken from the gums of cats (Carter, unpublished).

Morphology. P. multocida is generally described as a small rod or coccobacillus. Many cultures are markedly pleomorphic on initial isolation or when grown under unfavourable circumstances, e.g. reduced oxygen tension and in the presence of homologous antibody. Old laboratory cultures may be made up largely of long filamentous forms. Smith (1958) noted only slight differences in the morphology of strains recovered from the ox, pig, dog and cat.

There is only occasional reference to the presence of capsules in the older literature. Capsules may be demonstrated on all serotypes.

Three methods have been employed for the demonstration of capsules. They are, the method of Jasmin (Jasmin, 1945; Smith, 1958), the alcian blue method (Bain, 1953; Gurr, 1956), and the India ink method (Duguid, 1951).

Of the known serotypes the largest capsules have been observed on type A organisms (Carter, 1958c). These capsules are composed in part of hyaluronic acid (Carter & Annau, 1953, Bain, 1954a), and they impart a moist, mucoid appearance to colonies grown on enriched media. Staphylococcal hyaluronidase as well as bull testicle hyaluronidase inhibits the production of the large hyaluronic acid-containing capsule (Carter & Annau, 1953; Carter, 1957b). The capsules of the other three serotypes of P. multocida are considerably smaller. Absence of capsule is not always associated with low virulence for mice (Carter, 1957a), nor is the converse necessarily true (Sinha et al., 1957).

Smith (1958) examined 103 strains for the presence of capsules by Jasmin's method. Capsules or zones interpreted as capsules were observed on 36 of 103 strains. Three of the five ox cultures had capsules, 24 of the 28 pig cultures, five of the 39 dog cultures, and four of the 31 cat cultures. Bain (1957b) noted that all of 20 dog and cat strains were non-capsulated on primary isolation.

Variation. The colonial variation of P. multocida has been studied extensively. Much confusion has arisen because a variety of terms have been used by different workers to denote the same variants. It was suggested recently (Carter, 1957a) that the terminology suggested by Braun (1953) be adopted as a standard for this species. The various terms that have appeared in the literature over the years are listed in Table 1 along with the corresponding designations in the proposed standard nomenclature.

TABLE 1

Proposed and Older Designations for Colonial Variants *

Terms employed in the past	Proposed Terms	
	Colonial	Antigenic
Mucoid (Webster & Burn, 1926)	Mucoid	M
Fluorescent (Hughes, 1930) Diffuse (De Kruif, 1921)	Smooth	S
Intermediate (Hughes, 1930)	Smooth	S
Granular (De Kruif, 1921) Blue (Hughes, 1930)	Smooth	SR
Rough (Anderson et al., 1929)	Rough	R

* Carter (1957a)

Braun suggests that the terms mucoid, smooth and rough be employed to designate the principal colonial variants of bacteria. All of these variants are seen in cultures of P. multocida although rough colonies occur infrequently. The different variants can be identified with the low power of the microscope employing oblique light, and by the reaction produced by heavy suspensions of variants in acriflavine (Carter, 1957a). The three principal colonial variants are:

(1) Mucoid—these colonies are the largest. Many of the freshly isolated type A strains produce a preponderance of mucoid variants.

(2) Smooth (iridescent)—these colonies are smaller and discrete.

(3) Smooth (non-iridescent)—this variant resembles the one directly above except that the constituent organisms have less capsular substance. They are usually grey or greyish-blue. Some cultures are seen that are composed of a predominance of non-capsulated smooth variants (Carter, 1957a).

The relationship of the characteristics: typeability, acriflavine reaction and capsule to the different variants are summarized in Table 2. Braun has pointed out that "the acriflavine test has proved to be a far more reliable indication of antigen characteristics than mere observation of colony morphology". In this connexion it has been noted repeatedly that cultures whose variants produce strong flocculation in acriflavine do not possess sufficient capsular substance for type identification by the haemagglutination test. These cultures have been described as antigenically rough. Braun would designate such a culture as S^R (see Table 2).

TABLE 2

Some Properties of P. multocida and their Relationship to Colonial Variation *

Colonial Variant	Antigenic Designation	Capsule	Typeable by Haemagglutination	Acriflavine Reaction
Mucoid	M	++	Yes	Slimy
Smooth	S	+	Yes	Cells remain suspended
Smooth	S^R	–	No	Flocculation
Rough	R	–	No	Flocculation

* Carter (1957a)

It is of interest that freshly recovered capsulated strains of serotype D produce a characteristic coarse clumping in acriflavine. This reaction is sufficiently apparent and specific to make possible the rapid recognition of type D strains (Carter, 1957a).

The relationship of colonial variation to virulence for laboratory animals has been studied extensively. With the exception of the large mucoid variants which appear to be produced only by type A cultures, variation in the different serotypes is very similar. Although virulence for mice of the various serotypes is roughly comparable, virulence for other animal species varies greatly. It will suffice to summarize briefly the available information on the relationship between variation and virulence.

(1) Cultures initiated from mucoid variants vary greatly in their virulence for mice, e.g. many mucoid cultures from cases of acute fowl cholera are highly virulent for mice whereas mucoid cultures that tend to autolyse are weakly or moderately virulent sometimes taking as long as a week to kill mice (Carter & Bigland, 1953).

(2) Cultures initiated from smooth (iridescent) variants are almost invariably highly virulent for mice.

(3) As iridescence is lost mouse virulence may or may not drop. Many laboratory strains that yield smooth (non-iridescent) variants are highly virulent for mice (Carter, 1957a).

(4) Cultures that yield rough variants are low in virulence for mice (Anderson et al., 1929).

The term "phase I' employed by Bain (1954a), to describe the fully immunogenic state of type B is considered to be a functional rather than a morphological concept and is therefore not synonymous with smooth (iridescent). Bain & Knox (1960), found that smooth (non-iridescent or blue) variants of type B synthesized as much polysaccharide as the capsulated forms but were unable to retain it at the surface presumably because some linking substance was absent.

The account of variation presented above is a simplification. There are considerable differences amongst the various serotypes and each should be investigated separately.

Biochemical Activity. A number of attempts have been made to separate strains into groups on the basis of the fermentation of different compounds. Rosenbusch & Merchant (1939) observed that strains belonging to their serological Group I did not ferment xylose while the Group II strains did. The Group I strains fermented arabinose and dulcitol while the Group II strains did not. However, variations in this fermentative pattern were observed. Roberts (1947) found that his type B strains were all xylose positive, arabinose negative. This has since proved to apply to all except one of 70 strains of Asian type B from haemorrhagic septicaemia. The Australian atypical B (2 strains) did not ferment xylose and were pathogenic for chickens (Bain, 1957b, 1959). Das (1958) found no significant difference in fermentation amongst 49 strains collected from cattle, buffaloes, sheep, goats and fowls, nor any correlation between sugar fermentation and virulence for mice. Although there is some correlation between fermentative properties and serological groups no satisfactory division into groups has been accomplished as yet on this basis.

Smith (1959) observed that the fermentative properties of strains from dogs and cats are frequently different from the reactions obtained

with strains of bovine and porcine origin. He noted that many canine and feline strains split maltose, dextrin and lactose, whereas only a few bovine and porcine strains could utilize these compounds. The different biochemical activities of canine and feline strains as well as some other less well-defined characteristics indicate, as Bain (1957b) has suggested, that the strains found naturally in these species probably constitute a group or groups distinct from the recognized serotypes. This is also implicit in the findings of Talbot & Sneath (1960) using 59 strains from human infections.

Bergey's manual (Breed et al., 1957) refers to the production of hydrogen sulphide. Traces of hydrogen sulphide may be produced on prolonged incubation but it is difficult to detect with the media commonly used for this purpose (Carter, unpublished).

Generally speaking conventional methods are considered adequate for the isolation of P. multocida. Das (1958), and Morris (1958) have described selective media for the isolation of P. multocida from heavily contaminated material.

Nutrition. Several reports have appeared on the nutrition of various strains of P. multocida. Unfortunately, the investigators did not employ cultures of known serological type.

Jordan (1952a), following up Webster's (1925) observations, noted that in the cultivation of P. multocida blood could be replaced by haematin, catalase, sodium sulphite, and a number of compounds capable of catalysing the decomposition of hydrogen peroxide. However, blood and the compounds which would replace it were not required for anaerobic growth. She concluded that the mechanism of hydrogen peroxide decomposition did not necessarily account for the effect of haematin on growth because haematin had no effect on the production of the enzyme in stationary cultures. In a second report Jordan (1952b) noted that in a chemically defined medium glucose, maltose and galactose inhibited aerobic growth to a variable extent when added as the only carbon sources whereas lactic acid and sucrose were adequate sources of carbon.

Berkman et al. (1940) studied the requirements of several strains of P. multocida for some accessory growth factors. Nicotinamide and pantothenic acid were required for growth. In addition, several cultures required "the butyl factor" which the authors suggest was biotin. In a later report Berkman (1942) found that pure biotin would not replace the "butyl factor" but that a less pure biotin preparation would. It was noted that nicotinamide could not be replaced by nicotinic acid.

For cultivation of type A or B strains in aerated vessels by the batch or continuous process, a basal medium of casein hydrolysate is convenient. Yields are increased to 2 g. dry weight per litre by the addition of yeast extract and tryptone. The addition of individual growth factors has little effect (Bain & Jones, 1958). A potent stimulator which increases the yield to 4.5 g./litre is an auto-digest of pancreas (Sterne & Hutchinson, 1958). A fair substitute is pancreatic digest of liver (Bain, 1959). The specific substance has not yet been identified.

Serological Classification. Since the beginning of the century attempts

have been made to distinguish serological varieties of P. multocida. A number of serological procedures, including agglutination, complement fixation, and precipitation were employed and different varieties were recognized but no satisfactory classification or routine test for the recognition of types evolved.

The first serological classification to gain any measure of acceptance was that of Little & Lyon (1943). By means of the slide agglutination test they divided their strains into three serological groups designated types 1, 2 and 3. This method of identifying serological types has not been found satisfactory for various reasons (Carter, 1958a), and consequently is not being used. It is unfortunate that some workers have continued to use these type cultures in recent studies (Sinha et al., 1957; London & Yaw, 1957).

Roberts (1947) employed serum protection tests in mice to separate strains into four groups which he designated types I, II, III and IV. This grouping based on immunological differences gave clear-cut results. Roberts' method of typing P. multocida has not been employed widely because it is expensive and laborious. It is, however, the only method which at present will determine the serological type of strains that are colonially smooth but antigenically rough, i.e., strains, the organisms of which have little or no specific capsular substance. They must, however, be pathogenic for mice.

Ochi (1952) employed the tube agglutination test to separate serological varieties of P. multocida and designated his types A, B, C and D. He stated that his type A strains were isolated from birds, while his types B, C and D were isolated from cattle, pigs, sheep and rabbits. He stated also that his type B is the cause of epizootic haemorrhagic septicaemia of cattle.

Carter (1958a) attempted to type strains of P. multocida by the agglutination procedures described by Little & Lyon, and Ochi. He was unable to separate in a satisfactory way strains that had been shown to be different by serum protection tests in mice, and the haemagglutination test.

Strains were separated into three distinct types by means of a precipitation test in which the antigen consisted of a saline solution of capsular substance (Carter, 1952), and in view of this, it was stated that P. multocida possessed a type specific capsular antigen. For a number of reasons neither the precipitation test nor the capsular swelling technique lent themselves to routine use.

Several years later a haemagglutination test was evolved in which the specific capsular antigens were adsorbed on red blood cells (Carter, 1955). These treated red cells were then added to dilutions of the type specific immune sera. When sufficient capsular antigen was present haemagglutination was observed in the tubes containing the homologous antibody. By means of this test several hundred strains of P. multocida have been separated into four principal groups designated types A, B, C and D.

Roberts' types I and II correspond respectively to Carter's B and A. Roberts' types III and IV were equated with Carter's C and D respectively by means of the haemagglutination test (Carter, 1958). However, it is

deemed inadvisable to equate these latter types with Carter's types until additional serum protection tests are carried out. Because the methods of Rosenbusch & Merchant (1939), and Little & Lyon (1943) do not in our opinion reliably identify serological types, no particular purpose is served by equating these types.

Bain (1955a) was able to decapsulate Roberts' type II, III and IV with bull testicle hyaluronidase. This indicates the presence of hyaluronic acid in the capsules of these organisms. Hudson (1954) described an additional serotype from pigs which he designated type V in the Roberts' scheme.

The strains that have been identified as type A by the haemagglutination test vary greatly in their animal origin, colonial appearance and virulence for mice. The titres at which antigen-treated red cells are agglutinated vary greatly with different strains, indicating considerable serological variation. The possibility of the occurrence of immunological subtypes in this serological category has not been ruled out.

The type B strains studied make up a reasonably homogeneous group. Cultures that would appear to be variants of type B have been isolated from cases of haemorrhagic septicaemia in the Belgian Congo and French Cameroon (Carter, unpublished). A variant of type B has been observed in Australia (Bain, 1957b, 1959).

Type C cultures occur infrequently. It was thought at one time that canine and feline strains might fall into this category but so far these cultures have resisted classification. Smith's (1958) observations on canine and feline strains suggest that they differ considerably from many bovine and porcine strains. Bain (1957b) has also commented on characteristics which distinguish them from the known serotypes.

Type D constitutes a considerable proportion of the strains isolated from a wide range of animals. They appear to be more antigenic in rabbits than type A strains and possess considerably more antigenic stability (Carter, unpublished).

From the standpoint of disease, types A, B and D are by far the most important. Eight hundred and sixty-four cultures were subjected to the haemagglutination test. Three hundred were not typeable, and of these all except 29 gave reactions with acriflavine that indicated antigenic roughness, i.e., little or no specific capsular substance. The distribution of these non-typeable strains according to animal species is shown in Table 8. A number of as yet undesignated serological and immunological types have been identified.

It has been known for years that strains of P. multocida frequently yield bacteriophage. However, no success has so far attended efforts at typing with bacteriophage. Rifkind & Pickett (1954) found that of 118 strains, 84 were sensitive to one or more of 16 bacteriophages. Some of the cultures required calcium or magnesium ions as a co-factor for lysis in broth. Eleven of 25 avian cultures studied by Kirchner & Eisenstark (1956) were lysogenic.

The evolution of a satisfactory serological classification of this species has been fraught with considerable difficulty. This has been due to a great extent to the remarkable capacity for variation, the close

antigenic relationship of strains and the frequent occurrence of strains amongst the animals commonly employed for the production of immune sera.

TABLE 3

Relation between Serological Type of P. multocida and Animal Host *

Animal Host	TYPES			Not Typeable
	A	B	D	
Buffalo	2	13	0	0
Caribou	3	0	0	0
Cat	3	0	1	27
Cattle	88	23	6	47
Chinchilla	0	0	0	7
Chipmunk	0	0	0	1
Deer	0	1	1	0
Dog	0	0	3	14
Fowl	56	0	6	125
Goat	0	0	1	1
Guinea-pig	1	0	2	1
Horse	0	1	1	1
Man	20	0	11	16
Mink	3	0	1	10
Monkey	1	0	0	0
Mouse	0	0	2	3
Muskrat	1	0	0	2
Pig	157	3	118	19
Rabbit	11	0	3	13
Sheep	11	1	9	13
Totals	357	42	165	300

* Compiled from Carter (1955, 1957b, 1958b, and unpublished data).

Antigenic Structure. The various strains that compose the species P. multocida possess a number of common somatic antigens. This can be inferred from the behaviour of strains in the

simple agglutination test, and from the results of diffusion precipitin analysis (MacLennan & Rondle, 1957; Bain, unpublished; Carter, unpublished).

Serologically active polysaccharides were recovered from strains of P. multocida by Hoffenreich (1928) and Dingle (1954). Yusef (1935) observed the occurrence of serologically specific capsular substances in strains of P. multocida. Carter (1952) extracted soluble type specific antigens from the capsules by heating at 56°C. Carter & Annau (1953) published analyses of these type specific antigens which indicated they were polysaccharides. The comparatively high nitrogen content (over 5%) and the presence of phosphorus and pentose suggest contamination with nucleic acid which would be expected from the method of preparation. A polysaccharide which was depolymerized by bull testicle hyaluronidase was also found and provisionally identified as hyaluronic acid. Concurrently Bain (1953, cited by Hudson, 1959 but otherwise unpublished) isolated from two type II and one type IV strain a viscous polysaccharide containing equal amounts of a hexosamine and uronic acid. This was depolymerized by bull testicle and streptococcal hyaluronidase and although the specific components were not identified as N-acetyl glucosamine and glucuronic acid it was considered reasonable proof that the substance was hyaluronic acid. Because most of the work on antigenic structure was carried out with Roberts' types his designations will be employed in this section.

Bain (1955a) noted that the large capsule strains of types II, III and IV were readily decapsulated by bull testicle hyaluronidase, whereas type I was unaffected. Carter (1958c) has reported the decapsulation of type A strains by means of hyaluronidase produced by Staphylococcus aureus. Repeated analyses by Bain (1955a) have failed to show any uronic acid in extracts from type I. The presence of hyaluronic acid renders strains inagglutinable in homologous antiserum. These bacteria nevertheless absorb antibody from sera and when exposed to fluorescent antibody and examined by ultra-violet light, the whole thickness of the capsule is seen to fluoresce (Bain, unpublished).

From all types the gentlest saline extraction yields a multiplicity of antigens as revealed by agar-gel precipitin tests. It appears that in types II, III and IV the hyaluronic acid is disposed more as a framework than as a discrete layer. Other polysaccharides with high uronic, low hexosamine and the converse, high hexosamine and low uronic, were isolated in the same experiments. The extraction method which Amies (1951) used on P. pestis was tried using 0.5 M potassium thiocyanate but it failed to break down the capsules containing hyaluronic acid. When applied to type I it dissolved the capsules rather more slowly than those of P. pestis. The crude extract yielded 12 lines on Ouchterlony plates and was separable into polysaccharide, protein complex, and lipopolysaccharide antigens (Bain, 1955a and unpublished) by orthodox chemical and biophysical methods such as ethanol and ethylene glycol fractionation, isoelectric precipitation, ultra-centrifugation, and absorption and elution in columns. The polysaccharide obtained by simple saline extraction, even when freed from protein by Sevag treatment, tryptic digestion, reprecipitation in ethanol and precipitation by acetone from ethylene

glycol, still had a nitrogen value of about 7%. It was free from nucleic acid, and was not immunogenic in rabbits, mice or cattle although it removed some protective power for mice from immune sera. In its chemical and physical behaviour it resembled a mucopolysaccharide.

The crude protein fraction of type I consisted of several immunologically distinct substances including polysaccharide, lipopolysaccharide, nucleic acid, protective protein and protein toxin. It was difficult to resolve the complex into separate, chemically pure entities even by elaborate methods. The protein fraction, when freed from gross admixture with other components, was strongly immunogenic in mice and buffaloes (Bain, 1955a). Dhanda (1960) using conventional fractionation methods recovered a soluble protein antigen from type I which would appear to be similar to Bain's protein fraction. Stamatin (1958) also noted the effectiveness of protein antigens.

MacLennan & Rondle (1957) extracted type specific lipopolysaccharide antigens from type V and inferred the presence of similar substances in types I and IV. Bain & Knox (1960) also extracted lipopolysaccharide containing heptose from type I and this is the fraction which is adsorbed to red cells for the haemagglutination test. The colorimetric estimation of heptose was a reliable test for the presence of lipopolysaccharide, and the polysaccharide and protein fractions which were heptose-free failed to be adsorbed on red blood cells.

Laverty (1959) fractionated a type IV strain by saline extraction, isoelectric precipitation and phenol extraction. The general pattern resembled type I except that hyaluronic acid was present and that the yield of lipopolysaccharide was slight. He did not detect any polyuronides or hexosamines apart from the hyaluronic acid but he did find other hexoses, viz., glucose and galactose.

Bain (1954a) introduced a provisional term "phase 1" to describe the fully immunogenic state of type B. A simple and probably incorrect comparison was made with the "Vi" antigen of Salmonella. The 'phase 1" state may mean no more than the optimum content, distribution and physical state of important immunizing antigens expressed by the ability of the bacteria to protect cattle in low dosage. The term is not necessarily synonymous with 'fluorescent' which is a manifestation of the polysaccharide content of the bacteria.

The occurrence of heterophile antigens in strains of P. multocida has been reported by Dingle (1934) and Buchbinder (1934).

Toxin Production. Some of the toxicity of cultures of P. multocida can be attributed to the glycolipid or "Boivin antigens" first described by Pirosky (1938a, b, c). A protein toxin thought to be similar to that described by Ajl et al. (1955) for P. pestis has been identified from type B cultures of P. multocida by Dhanda (1960) and also by Knox & Bain (1960).

Bain (1959) noted that in type B, toxic pyrogenic lipopolysaccharide was present in even the preliminary saline washings from harvested bacteria. Laverty (1959) found the same in type A. The substance was measured by its heptose content. In current experiments doses of up to 4 mg. of purified lipopolysaccharide have failed to kill young buffaloes

although they have shown pyrexia and malaise. The filtered plasma of experimentally infected cattle, taken when the animals were moribund, has produced hypothermia in mice and hyperthermia in rabbits, suggesting the presence of lipopolysaccharide. It is probable that both the toxic lipopolysaccharide, recovered from crude capsular extracts, and the protein cause the symptoms leading to death (Bain, 1955a).

Smith (1957) demonstrated the production of what he termed a toxin in 5 of 10 strains of P. multocida. Seven-day-old broth cultures contained 20 to 160 mouse lethal doses per ml. Occurrence of toxin in broth cultures was associated with lysis of the bacterial cells. The toxin was inactivated by heat (10 min. at 60°C.), treatment with acid and 0.5% formalin. It was resistant to proteolytic enzymes and antigenic in rabbits. No relation between toxin production and virulence or infectivity for mice could be demonstrated.

Distribution of Serotypes in Animals. The wide occurrence of type B strains in haemorrhagic septicaemia of cattle and buffaloes was noted by Bain (1954a) as a result of typing 39 bovine strains collected in six Asian countries. Hudson (1954) typed 15 cultures from cases of haemorrhagic septicaemia in three Asian countries and also from Kenya. He concluded independently that haemorrhagic septicaemia of bovines was caused by type B. Since 1952 Bain has examined 120 cultures from cases of bovine haemorrhagic septicaemia in Asia or stock cultures from Asian laboratories. Countries of origin were Egypt, Iraq, Iran, Pakistan, India, Ceylon, Burma, Thailand, Malaya, Indonesia, Indo-China, and the Philippines. All were type B by the mouse protection test. Further evidence of the wide occurrence of type B in cattle is given in a recent report by Dhanda (1959).

Eight hundred and sixty-four cultures from 20 different animal species were examined by Carter. The distribution of types A, B and D for each animal species is shown in Table 3 (p. 114). The most prevalent type from cattle and fowls was type A. This type was also the most frequent type from pigs, although almost as many type D cultures were identified from this species. The largest number of type B cultures were from cattle and buffaloes. Of the 31 typeable cultures of P. multocida from man, 20 were type A and 11 type D. The number of cultures from other species was perhaps not large enough to give a reliable indication of the occurrence of types.

Geographical Distribution of Serotypes. Types A and D are world-wide in distribution (Carter, 1957b, 1958b). Epizootic haemorrhagic septicaemia of cattle and buffaloes does not appear to have occurred in North America for at least the last three decades. Type B cultures recovered from outbreaks of haemorrhagic septicaemia of buffaloes in the U. S. A. are still in existence. Type B strains occur throughout Asia, in Africa, and in Southern Europe. Although strains have been isolated in England and Australia that resemble type B, haemorrhagic septicaemia has not been reported as an epizootic in these countries in recent years.

There are, no doubt, a large number of serological types. Roberts

(1947) suggested that the distribution of pasteurella serotypes may resemble that of the salmonella group, in that there probably are a small number of frequently occurring types and a large number of less frequently occurring types. There are already indications that types as yet undesignated occur in geographical areas that are somewhat remote.

Distribution of Serotypes in Infections. The data presented below, except where stipulated, are drawn from reports published recently by Carter (1955, 1957b, 1958b, and unpublished).

Type A: This serotype is the principal cause of fowl cholera, and it is also frequently found complicating virus or enzootic pneumonia of pigs. Of 135 cultures isolated from lesions of pneumonia in pigs 77 were type A (Carter, 1957d). The majority of type A strains recovered from cattle were isolated from pneumonic lungs (Carter, 1957c). This type was recovered from a wide variety of sporadic infections in many different animal species (see Table 3).

Type B: This type has been established as the cause of epizootic pasteurellosis or haemorrhagic septicaemia of cattle and buffaloes (Bain, 1954a; Hudson, 1954). There have been occasional reports of the isolation of this species from animals other than cattle and buffaloes. Type B strains were identified from Spanish pigs by Roberts (1947), and from fatal pasteurellosis in horses in Egypt (Geneidey, 1955). Nikiphorova (1958) records the recovery of Type B from sheep in Russia.

Type D: Of 135 cultures examined from pneumonic lesions in pigs 57 were type D (Carter, 1957d). This type has been frequently recovered from a variety of sporadic infections in a wide range of species (see Table 3).

Type C: This type appears to constitute a small group of no special importance in disease. Only one strain of Roberts (1947) type III which is considered identical to type C was identified in a recent survey by Dhanda (1959).

Of the 300 cultures that could not be typed, all except 23 gave reactions with acriflavine that indicated antigenic roughness, i. e., little or no specific capsular substance. The significance of the isolation of these cultures from various animals is difficult to assess. It is doubtful if these comparatively avirulent strains are often the primary cause of disease.

Infections: General. By virtue of its ubiquity and capacity for opportunism, P. multocida is found in a wide range of infections in animals. It has been known for many years that outbreaks of acute disease caused by P. multocida are usually precipitated by circumstances which result in physiological stress. The ensuing infections are attributed to a combination of increased susceptibility of the animals and heightened virulence of the organisms though this has only rarely been proved. Cavallero & Sala (1951) have shown that latent infections of P. multocida in rats are made acute and fatal by inoculating cortisone, a substance released in relatively large amounts during stress.

The principal diseases of animals caused by P. multocida, either wholly or in part, are: epizootic pasteurellosis of cattle or haemorrhagic

septicaemia, shipping fever or stockyards pneumonia of cattle, pneumonias of sheep and goats, fowl cholera and secondary pasteurella pneumonia of swine. In acute fowl cholera and haemorrhagic septicaemia of cattle P. multocida appears to be the sole causative agent. In the other diseases referred to above the aetiology is frequently complex, e.g. although P. multocida may occur in shipping fever, P. haemolytica is more important (Carter, 1956). Viral agents are no doubt involved in the aetiology of most of the pneumonias of domesticated animals. This, however, does not mean that the presence of P. multocida as a secondary invader is of no significance.

Daubney et al. (1934) suggested that fleas may have spread infection amongst calves in Kenya. However, it is generally considered that natural infection is via ingestion or inhalation. The epidemiological factors in haemorrhagic septicaemia have been discussed by Vittoz (1952), Bain (1957a) and Pillai & Thambiayah (1957).

Sporadic infections are well known in the diagnostic laboratory, two that have been recognized with greater frequency in recent years are mastitis in cattle and involvement of the central nervous system in several species.

There are several reports in the literature of mastitis of cows caused by pasteurella. Barnum (1954) described a herd outbreak of mastitis caused by this species. The mastitis was severe, resulting in abnormal secretion, loss of function, atrophy, and fibrosis of the affected quarters. That pasteurella mastitis is more prevalent than is generally appreciated is suggested by the fact that during one year (April 1959 to March 1960) 40 isolations were made constituting 0.5% of the quarters with mastitis (Barnum, 1960). Only 51 isolations of Corynebacterium pyogenes were made that same year from quarters with mastitis. The evidence suggests that pasteurella infecting udders is acquired from unweaned calves.

Several reports of the isolation of pasteurella from the brains of cattle and horses have appeared. Shand & Markson (1953) describe an outbreak of meningo-encephalitis in calves. P. multocida was isolated in pure culture from the three calves examined. An organism fitting the description of P. multocida was isolated from the brains of cattle dying from encephalitis (Rose & Rac, 1957). Byrne (1960) recovered P. multocida from the brains of four young pigs that displayed symptoms indicating damage to the central nervous system.

Infections in Human Beings. Reports of infections due to P. multocida have appeared occasionally in the literature since 1913 (Lévy-Bruhl, 1938). Within the last decade there has been a considerable increase in the number of isolations reported. Byrne et al. (1956) state that between 1947 and 1956, 45 cases were reported. This wider recognition of human infections is no doubt due to an increasing familiarity with this species.

Probably the most frequent and best known of the infections in man due to P. multocida are those resulting from animal bites, particularly those of the cat and dog. Infections of the respiratory tract occur next in frequency. In those infections of various tissues of the body not initiated

by animal bites, the portal of entry is not known although it is surmised that it is *via* the digestive or respiratory tracts.

It has been suggested that *P. multocida* may be a commensal in the upper digestive and respiratory tracts of man. If this were frequently the case one would expect that this organism would be recovered more often from throat swabs, and found more frequently as an opportunist in various pathological processes. It has been noted that it can survive in the human upper respiratory tract for many months (Wilson & Miles, 1946).

Pasteurella organisms found in the upper respiratory or digestive tract of man are very likely acquired from cattle, pigs, fowls, dogs, cats and other species. Many domesticated animals, both healthy and sick, must discharge considerable numbers of *P. multocida* in their exhalations and secretions. The numbers of pasteurella organisms discharged *via* the nostrils when cattle have pneumonia in which *P. multocida* is involved could be large. Twenty-seven of the thirty-seven patients studied by Olsen & Needham (1952) were either farmers or members of farmers' families. That many of the infections were derived from animals is also suggested by the fact that of 31 typeable cultures of human origin examined by one of the authors (G.R.C.) 20 were type A and 11 were type D (see Table 3). These serological types are the most prevalent in farm livestock other than the cat and dog.

The pattern of infection following a bite is described by Byrne *et al.* (1956) as follows: "Soon after the bite, the area becomes painful, red, tender, and markedly oedematous, with an ascending lymphangitis and lymphadenitis. In the absence of antibiotic therapy an abscess almost always develops at the site of the bite which heals slowly."

Septicaemias have only been rarely reported (Lévy-Bruhl, 1938). Of the respiratory infections reported bronchiectasis, and bronchiectasis with empyema are the most frequent (Olsen & Needham, 1952). Lévy-Bruhl dealt in detail with 19 human cases. They were in order of greatest frequency:

Pleurisy	4
Meningitis	4
Cat bite	3
Bacteraemia or septicaemia	2
Pleurisy, rheumatism and pericarditis	1
Post-partum infection	1
Pyomyositis	1
Conjunctivitis	1
Pneumonia	1
Panther bite	1

Besides 27 cases of bronchiectasis, Olsen & Needham recovered *P. multocida* from chronic bronchitis, peritonsillar abscess, appendiceal

abscess and infection of a gouty joint. Other infections yielding P. multocida are sinusitis (Olsen & Needham) peritonitis (Coghlan, 1958) and brain abscess (Svendsen, 1947). It is impossible to say how many of these conditions were primary pasteurella infections. Those resulting from animal bites may be considered primary. The results of treatment of these infections with antibiotics effective against P. multocida suggest that this species has a considerable pathogenic potential for man (Olsen & Needham). As yet there have been no reports of isolation of type B strains from disease conditions in man.

Experimental Infections. The great majority of capsulated strains isolated from cases of acute pasteurellosis are highly pathogenic for mice and rabbits. For example, cultures from cases of acute fowl cholera (type A) and haemorrhagic septicaemia (type B) may require less than 100 organisms to kill a mouse. There is, however, a great variation in pathogenicity for mice of cultures isolated from less severe infections, e.g. chronic fowl cholera and various pneumonias (Carter & Bigland, 1953).

Smith (1958) observed that most dog strains possess low virulence for mice while cat strains possess considerably greater virulence. Carter (unpublished) noted that although feline cultures kill mice in high dilutions they do not kill as rapidly as many cultures of known types.

Types A and D organisms will readily kill fowls in small doses either by oral or parenteral routes. Although fowls are refractory to type B, cattle and buffaloes are killed by intravenous or subcutaneous inoculation of approximately 20,000 organisms. Bain (1954b) injected the bovine 'Insein' strain (type B) into 13 pigs. Death occurred only in those receiving doses greater than 0.1 ml. of undiluted 6-hour broth culture, i.e., more than 10,000 buffalo lethal doses.

Two of four experimental ponies in Thailand died from subcutaneous injection of 1.0 ml. of undiluted broth culture of the "Insein' bovine strain (Bain, 1954b). In the same experiment ten goats and three sheep showed no ill effects when given the same dose.

Mice, rabbits and fowls can be infected with virulent type A organisms by the oral route. Mice and rabbits may be killed when virulent type B organisms are administered orally (Carter, unpublished).

Immunization. There is an abundant literature on the immunization of animals for the prevention of the different pasteurelloses. At present there are four diseases due wholly or in part to P. multocida where either active or passive immunization might be employed on a large scale. These diseases are shipping fever or stockyards pneumonia of cattle, fowl cholera, secondary pasteurella pneumonia of swine (VPP, enzootic or infectious pneumonia), and haemorrhagic septicaemia of cattle.

Immune sera have been prepared in horses and cattle by the inoculation of dead and living cultures of pasteurella. These have been used in North America for decades for the prevention and treatment of shipping fever or stockyard's pneumonia. This disease for years was incorrectly termed haemorrhagic septicaemia. The popularity of these

products has waned greatly in recent years and many laboratories have discontinued their preparation. The lack of efficacy of these sera must be attributed partly to failure to select the most prevalent serological types of P. multocida and the exclusion of P. haemolytica. Also the indications for the use of these sera were frequently too broad.

Many vaccines and bacterins have been employed over the years for active immunization. The tissue aggressins and artificial aggressins were in vogue for a number of years but are no longer used. Live cultures have been used off and on since Pasteur's day but because of a tendency to instability and difficulties in distribution this form of vaccine has been abandoned. Hudson (1954) describes the production and testing of variants in mice and buffaloes.

Broth bacterins are in use for the prevention of shipping fever, swine pneumonia, and fowl cholera. Carter (1957c) suggested that the shipping fever bacterin for use in Canada should be prepared from type A strains of P. multocida and strains of P. haemolytica; and that the swine pneumonia bacterin be prepared from P. multocida, types A and D (Carter, 1957d). The efficacy of these bacterins has not been tested because of the difficulty of producing experimentally the less acute pasteurella infections. In view of Bain's extensive work and the generally conceded value of oily adjuvants in the elicitation of immunity against bacteria, the addition of adjuvants to all pasteurella bacterins is indicated.

Broth bacterins have not been effective in the prevention of fowl cholera. Visit (1958), employing chick embryo, alum precipitated and oily adjuvant vaccines, was not able to demonstrate appreciable immunity six to eight weeks after vaccination. Considerable immunity can be elicited with various vaccines but protection is of short duration. Heddleston and his colleagues claim effective immunity up to nine months for their adjuvant vaccine (Heddleston & Hall, 1958; Heddleston & Reisinger, 1959). Bain (1953) used oil-adjuvant vaccine in ducks and also bacteria decapsulated with hyaluronidase. immunity was good at the fourth week but waning by the sixth. Chick embryo vaccines elicit considerable immunity although its duration may be short (Carter, 1950; Dougherty, 1953). The principal pasteurella constituent in vaccines for the prevention of fowl cholera should be type A strains.

Much work has been done by Bain and his colleagues toward the development of an effective vaccine for the prevention of haemorrhagic septicaemia of cattle and buffaloes. Knowledge relating to immunization against this disease is much more detailed and advanced than is the case with the other less spectacular pasteurella infections.

Laverty (1959), working in Bain's laboratory, obtained poor yields of lipopolysaccharide from two type A strains in contradistinction to the good yields from type B. This might explain the comparatively poor persistence of immunity against type A in view of the known enhancing effect of P. pestis lipopolysaccharide on its protective protein antigens.

Until about 1950 vaccines against haemorrhagic septicaemia were mainly simple broth bacterins. The value of higher dosage was shown by Govinda Rau & Govil (1950) using agar-grown bacteria but this increased the risk of shock after injection. A vaccine consisting of lysed bacteria in saponin was introduced by Deipy & Mir Chamsy (1949). Good results

have been claimed for it in Iran but it has not been used much elsewhere because it is not suited to large-scale production and causes some undesirable local swelling after inoculation. In Burma, in 1952, Bain developed an oil-adjuvant vaccine based on liquid paraffin-lanoline without added mycobacteria which avoided the severe tissue reactions of the type described by Scott & Ginsberg (1959) in rinderpest vaccine. In limited field trials and in extensive laboratory tests the oil-adjuvant vaccine has conferred solid immunity for up to 12 months (Bain, 1954a, b, 1956, 1959; Iyer, Gopalakrishnan & Ramani, 1955; Dhanda et al., 1956; Dhanda & Lall, 1958; Iyer & Ranga Rao, 1959).

The so-called "Indian" and "Bain" oil-adjuvant vaccines mentioned by Dhanda et al. are similar, the only difference being in the strain employed and minor variation in the medium. Difficulties in producing the vaccine in bulk have been overcome by aeration techniques (Bain & Jones, 1955, 1958; Sterne & Hutchinson, 1958).

Useful results from alum-precipitated vaccine in cattle are claimed by Iyer et al. (1955). Bain (1957a) suggested the use of an initial dose of oil-adjuvant vaccine followed by annual injections of alum-precipitated vaccine should it transpire that tissue damage follows repeated injections of oil emulsion.

Type B strains may be very sensitive to the nutritional environment. Most strains dissociate readily and to phenotypic variability is added genotypic variability. Bain has recommended as an empirical measure that for production of vaccines against haemorrhagic septicaemia the seed should be kept in the form of frozen or dried infected blood to minimize the risk of dissociation.

Methods for assessing the immune response in cattle are the agglutination, mouse-protection, agar-agglutination and complement-fixation tests (Bain, 1954d, 1955b, 1957a). These have to be carefully standardized (Bain, 1960b).

Formalin is used almost universally in the preparation of pasteurella vaccine. Knox & Bain (1960) found that formalin binds the protein and polysaccharide elements in extracts of type I so that they cannot be separated by the usual methods. This may explain the beneficial effect in vaccines and also the inagglutinability by serum of formalin-treated type I suspensions (Bain, 1954c).

Post-Vaccination Shock. Shock following the inoculation of pasteurella organisms has been a problem incidental to vaccination for many years. Murphy (1956) has described the clinical features of post-vaccination shock in cattle in some detail. It has been recognized in Asia for several years, particularly since the advent of improved type B vaccines. It has occurred with agar-grown and broth-grown bacteria, alum-precipitated vaccine and lately with oil-adjuvant vaccine which had been blended rather coarsely (Bain, unpublished). Cattle and buffaloes of all ages are affected. The incidence is erratic but not high, probably about 1%. That the post-vaccination reaction is an anaphylactoid shock is suggested by the favourable response to anti-histamine drugs and epinephrine. It is of interest also that mice vaccinated with type D organisms were highly sensitive to small amounts

of histamine (Carter, unpublished). That other serotypes would similarly sensitize mice to histamine seems very likely.

Chemotherapeutic Agents. There is a vast literature on the effect of chemotherapeutic agents on P. multocida. An attempt will be made to present the essential facts with the full realization that by no means all of the references have been included.

The in vitro activity of the sulphonamides against P. multocida has received little attention. Employing the "tube-dilution" technique Neter & Gorzynski (1950) noted that sulphadiazine inhibited the growth of eight strains. It is well known in diagnostic laboratories that the growth of many strains is inhibited by the "triple-sulpha" disks containing sulphadiazine, sulphadimidine, and sulphamerazine.

A number of sulphonamides have been found effective in the treatment of pasteurellosis. Because of the difficulty of producing this disease experimentally much of the evidence for the efficacy of chemotherapeutic agents must of necessity come from clinical trials where a precise diagnosis may be difficult. Sulphaquinoxaline (Eveleth et al., 1949), sulphadimidine and sulphamerazine (Francis, 1947) reduced the severity of what would appear to have been, according to our present knowledge, virus pneumonia of pigs complicated by a pasteurella infection.

Sulphaquinoxaline, sulphadimidine and sulphamerazine were notably effective in the prophylaxis of experimental fowl cholera, but sulphadiazine, sulphathiazole and sulphanilamide were much less effective (Peterson, 1948). Sulphaquinoxaline was the most effective. Several workers (Delaplane, 1945; Peterson, 1948; Hilbert & Kiser, 1948) observed that although sulphonamides will check losses during the period of treatment, losses continue as soon as treatment is stopped. Sulphonamides do not appear to promote the resolution of chronic infections.

Sulphadimidine has proved curative if used very early against haemorrhagic septicaemia of cattle in Asia (Gopalakrishnan et al., 1957). Shirlaw (1957) tested eleven sulphonamides in vitro for activity against five strains. He found that only sulphathiazole had a definite inhibitory effect, while sulphamerazine, sulphadiazine and sulphadimidine, in that order, showed a moderate degree of inhibition only at the higher concentrations. Slavin & Maclay (1947) studied the effect of sulphonamides on pasteurella infections in mice. They found sulphadimidine and sulphadiazine more effective than sulphapyridine and sulphathiazole. Sulphanilamide was relatively ineffective.

The antibiotics that are highly active against P. multocida are listed in Table 4. The range of the minimal inhibitory concentration is given with the number of strains tested. Of the numerous sources of the data listed in Table 4 only several are referred to because of limitations of space.

Those antibiotics to which most strains were resistant in vitro were bacitracin (Neter & Gorzynski, 1950; Muysson & Carter, 1959), viomycin (Gorzynski & Neter, 1951; Muysson & Carter, 1959) and "Oxamycin" (cycloserine) (Cuckler et al., 1955).

Of the antibiotics that are active against P. multocida in vitro only a few have been employed for the prophylaxis and treatment of natural and

TABLE 4

Antibiotics showing in vitro Activity against P. multocida

Antibiotic	No. of Strains Tested	Range of Minimal Inhibitory Concentration (μg./ml.)	References
carbomycin	11	1–10	Gorzynski & Neter, 1953 Welch et al., 1952
cathomycin	3	0.78–3.12	Frost et al., 1955–56 Wilkins et al., 1956
chloramphenicol	29	0.25–10	Neter & Gorzynski, 1950 Muysson & Carter, 1959
chlortetracycline	25	0.1–3.12	Neter & Gorzynski, 1950 Muysson & Carter, 1959
erythromycin	24	0.2–10	Gorzynski & Neter, 1953 Muysson & Carter, 1959
furazolidone	5	0.4–6.1	Yurchenco et al., 1955
neomycin	19	6–25	Neter et al., 1951 Muysson & Carter, 1959
oxytetracycline	22	0.2–3	Neter & Gorzynski, 1950 Muysson & Carter, 1959
penicillin G	56	0.08–12.5 units	Schipper, 1947 Neter & Gorzynski, 1950
polymyxin	28	2–64	Stansly et al., 1947 Neter & Gorzynski, 1950
streptolydigin	1	6.2	Deboer et al., 1955–56
streptomycin	104	1–50 units	Coles, 1948 McNeil & Hinshaw, 1948

experimental pasteurellosis. Chlortetracycline (Alberts & Graham, 1951), penicillin (Queen & Quortrup, 1946) and streptomycin (McNeil & Hinshaw, 1948) were of value in fowl cholera. Kelton (1952) found in Burma that chortetracycline administered intravenously and sufficiently early was an effective treatment for haemorrhagic septicaemia of cattle. Other reports on in vivo action dealt with experimental infections in mice (Gorzynski & Neter, 1951; Kiser et al., 1953-54; Frost et al., 1955-56).

Weil et al. (1957) administered combinations of antibiotics, two at a time, to mice infected experimentally with P. multocida. They showed

that the effect of penicillin plus tetracycline was an additive one, but small amounts of erythromycin interfered with the action of both penicillin and tetracycline.

It would appear that strains of P. multocida do not develop resistance to antibiotics with as great a facility as do some other pathogens. Considerable resistance to streptomycin has been reported in two strains (Gorzynski & Neter, 1951; Stansly et al., 1947).

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